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**Mucous Flow and Ciliary Activity  
in the Trachea of Healthy Rats  
and Rats Exposed to  
Respiratory Irritant Gases**

(SO<sub>2</sub>, H<sub>3</sub>N, HCHO)

A functional and morphologic  
(light microscopic and electron microscopic)  
study, with special reference to technique

BY

TORRE DALHAMN

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STOCKHOLM 1956

FROM  
HEALTH

ACTA PHYSIOLOGICA SCANDINAVICA · VOL. 36 SUPPLEMENTUM 123

FROM THE DEPARTMENT OF OCCUPATIONAL HEALTH, THE NATIONAL INSTITUTE OF PUBLIC HEALTH, STOCKHOLM, AND THE DEPARTMENT OF ANATOMY, KAROLINSKA INSTITUTET, STOCKHOLM

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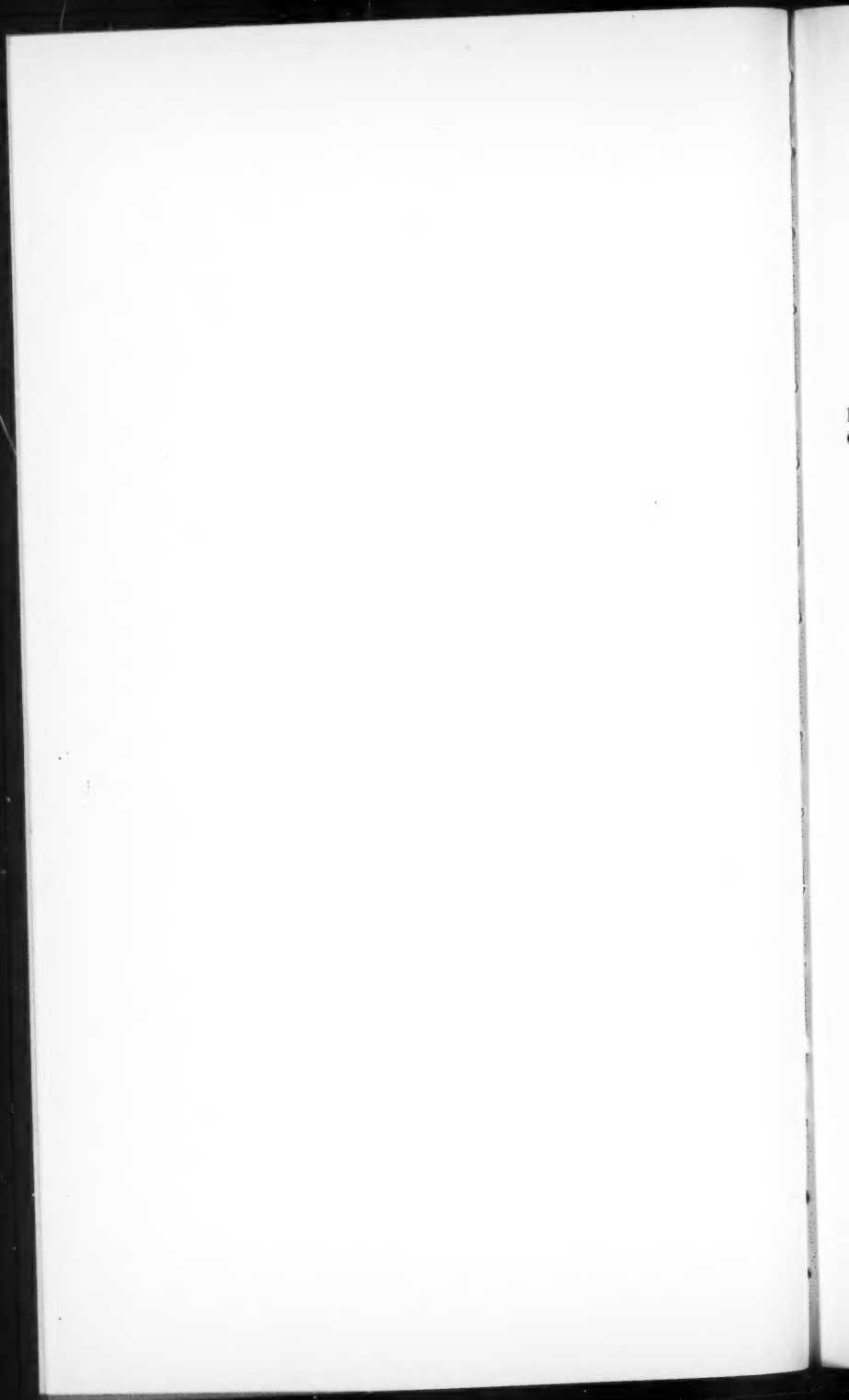
***TORRE DALHAMN***

STOCKHOLM 1956

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ESSELTE AKTIEBOLAG  
STOCKHOLM 1956

*To my wife*



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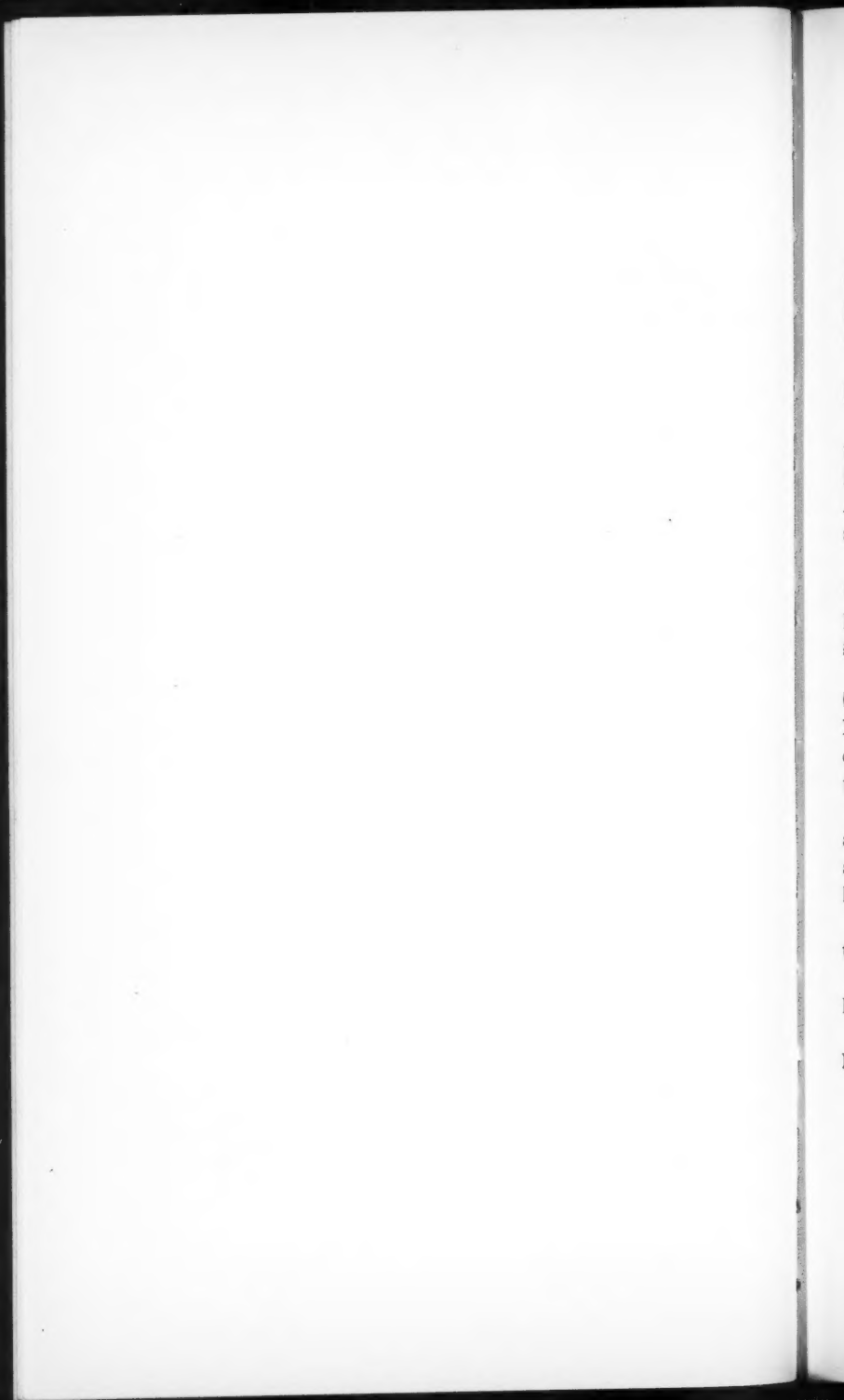
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## Preface

This investigation was carried out under the guidance of Professor AXEL AHLMARK, Head of the Department of Occupational Health, the National Institute of Public Health, and Associate Professor FRITIOF SJÖSTRAND of the Department of Anatomy, Karolinska Institutet. To both I extend my warm thanks.

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For interesting views on the subject under investigation and for many stimulating discussions I thank Professor SVEN FORSSMAN, Docent LARS FRIBERG, Docent JOHANNES RHODIN and Docent ÅKE SWENSSON, the last-named of whom suggested the plan of this study.

The Department of Photography at the Royal Institute of Technology, under the direction of Professor HELMER BÄCKSTRÖM, provided me with photographic equipment and Mr. ARTUR BOSTRÖM gave me much valuable assistance with the photographic technique.

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The great interest shown by Miss CHRISTEL ESROM in her laboratory assistance merits my deepest appreciation. Photographic assistance, particularly with the electron micrographs, was given by Miss MAJ BERGHMAN and Mrs. CHRISTINA LINDGREN.

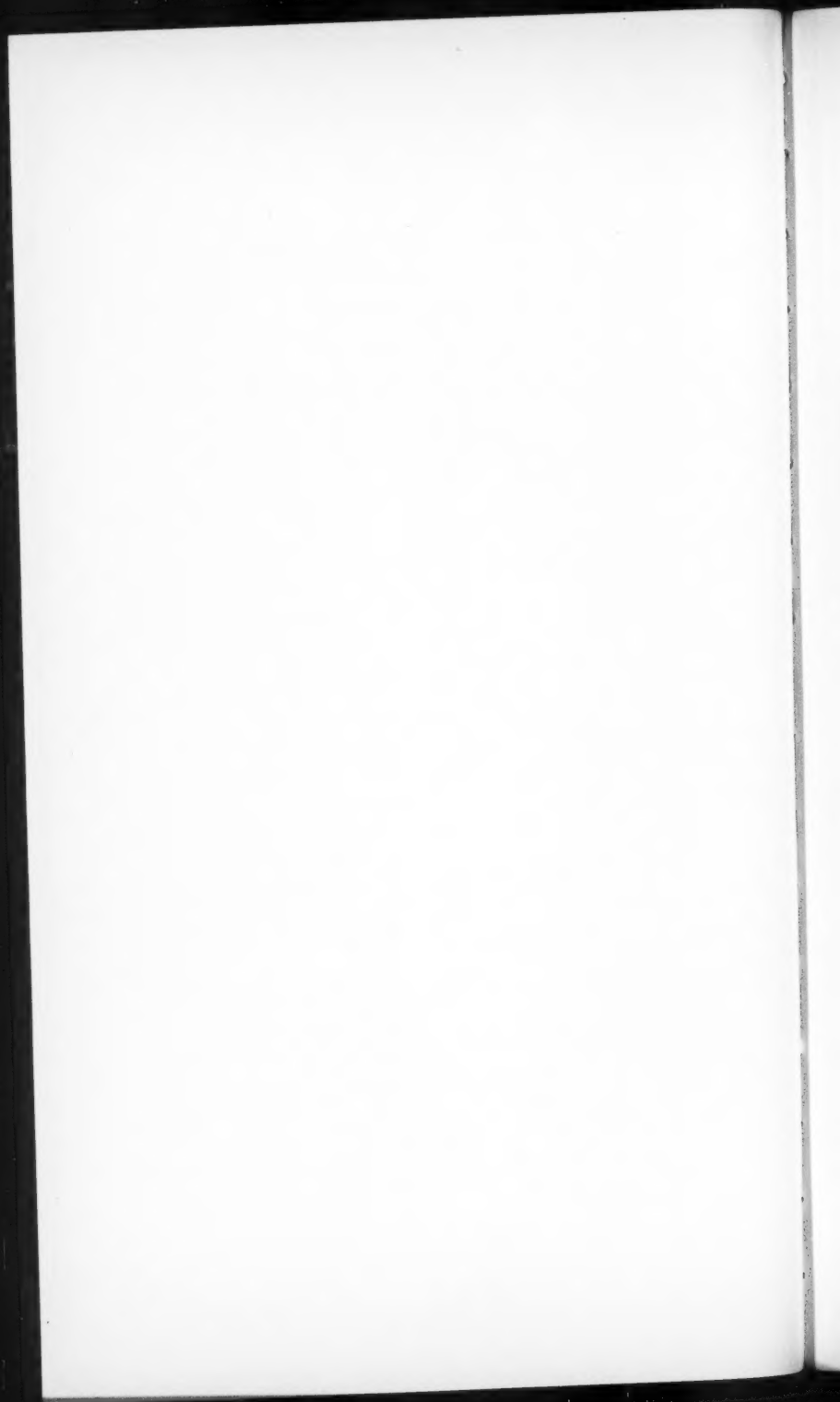
My thanks are also due to Mr. CARL-ERIC HOLMQVIST M.Sc. for technical advice.

To Mr. RUNE CEDERLÖF for statistical collaboration and to Miss RAY McVEIGH for the translation into English I express my thanks.

The investigation was financially facilitated by the Occupational Medical Research Grant.

Stockholm, February 1956.

*Tore Dalhamn*



## General Introduction

Cilia are phylogenetically and ontogenetically very early structures. They are present in protozoa and molluscs as well as in vertebrates. Their function varies greatly. In bacteria and protozoa cilia may be the organs of locomotion. The cilia of such animals as sea anemones assist nutrition by entrapping food from the surrounding water. Mussels and certain other marine forms are provided with gill cilia which propel water through the gills and thus promote oxygen uptake. Direct contact with a fluid medium is essential for ciliary function. In the above-mentioned marine forms the cilia are surrounded by water. Many land animals, however, are equipped with cilia, from lower species such as worms and larvae up to mammals. Higher animals, in contrast to water inhabitants, must themselves provide the fluid environment of their cilia. Several mammalian organic systems are ciliated, e.g. certain parts of the reproductive organs and most of the respiratory tract. These cilia are engaged in the transportation of such substances as gonadal products and respiratory-tract secretions.

The role of the cilia in the respiratory tract is exceedingly important. Thus the natural defence mechanism of the respiratory mucosa against harmful extraneous agents is dependent on the unremitting activity of the cilia and the resultant continuous transportation of mucous secretion. The composition of the mucus produced by the secretory cells of the mucosa and the deeper-lying glands is also of decisive importance for the efficiency of this protective mechanism.

Slowing of the mucous flow due, for instance, to altered composition of the secretion, may occur while the ciliary activity is unimpaired. For an understanding of the functional conditions, therefore, it is particularly important to be able to measure both mucous flow and ciliary activity. The consequences of retardation or total stoppage of mucous transportation are outside the scope of the

present study. The clinical significance is manifest. Elucidation of the aetiology, however, has hitherto been gravely hampered by lack of a method for quantitative determination of mucous flow and ciliary activity under normal and pathologic conditions. The author has evolved a new method for such determinations. With this method normal and certain pathologic conditions have been investigated.

Further knowledge of mucous flow and ciliary activity may be gained from observations of the normal and pathologic morphology of the tracheal mucosa carried out parallel with the functional studies. In the morphologic studies of the present investigation most importance was attached to ultrastructural findings. The structure of the cilia received special attention.

## I. Literature

Before reviewing earlier methods for studying mucous flow and ciliary activity in the respiratory tract of various animals, it should be pointed out that the above-mentioned mechanism for maintenance of normal conditions in the airways has been attributed very great importance by many writers, e.g. Barnett (1933), Negus (1934), Carlson and Johnson (1937), Leasure (1941) and Proetz (1946).

This significance was demonstrated by functional and morphologic studies of pathologic tissue in infections such as sinusitis and bronchitis. Of these reports may be mentioned Yates (1924), Florey, Carleton and Wells (1932), Latta and Schall (1934), Hilding (1943), and Wright (1950).

### *Studies of function*

#### **In vivo and in vitro experiments**

Many studies of mucous flow and ciliary activity have been carried out on extirpated membranes. Some writers, however, have emphasized the uncertain and unrepresentative character of the findings, e.g. Lommel (1908), Proetz (1933), Lucas and Douglas (1935), and Gordonoff and Mauderli (1936). Some experimental confirmation of these opinions has also been obtained. Thus Barclay and Franklin (1937) placed excised lung-trachea preparations from cats into Ringer's solution at 37° C and injected india ink into a bronchiole 1 mm in diameter. The time taken for the ink to be transported up through the trachea was recorded. The mean speed of flow in these *in vitro* experiments was 2.5 mm per minute in the lungs and 10 mm per minute in the trachea. The total distance in the lungs and trachea was traversed in about 25 minutes.

The *in vivo* experiments of Barclay and Franklin were made on decerebrate cats. India ink was injected subpleurally into the lungs and its appearance in the subglottic region observed through a cannula. In the two experiments the ink was carried from the lungs to the larynx in 14 minutes.

In the present connection it may be mentioned that in experiments with the extirpated trachea of dogs Lommel (1908) found the "optimal" speed of mucous flow to be 19.8 to 24 mm per minute, while v. Gebhart (1909) recorded a maximum flow rate of 12 mm per minute.

Ciliary beat frequency has not been compared *in vivo* and *in vitro*. The only comparative studies of ciliary activity concerned the effect of various applied agents. Lucas (1933 a) and Lierle and Moore (1935) found this effect to be more rapid and more pronounced *in vivo* than *in vitro*.

Because of the above-mentioned findings, only *in vivo* experiments will be referred to in the following discussion on *mucous flow*. Methods for determining *ciliary beat frequency* will be discussed irrespective of whether they were employed *in vivo* or *in vitro*. These methods are of special interest for the present study because of their technically complicated character.

#### **The rate of mucous flow in healthy animals**

As far as can be judged from the available literature, all estimations of the speed of mucous flow have been made by placing, e.g. particles of charcoal, graphite ink or pollen dust on the mucosa. In these experiments it was difficult to avoid coalescence of the particles. Writers such as v. Gebhart (1909), Henderson and Taylor (1910) and Hill (1928) have pointed out that small particles are transported more rapidly than large conglomerations. In the light of this finding the use of such transportation indicators must be considered unsatisfactory.

The earliest observations of mucous flow in the respiratory tract were made by Sharpey (1830), from experiments on the nasal mucosa of living rabbits. Animal charcoal was applied to the membrane and the movements of the particles studied. Since then several similar investigations have been described, some of them in

human subjects. Of these latter may be mentioned the studies of Yates (1924), Hilding (1931), Herrman (1933), Ornston (1946), and Tremble (1947, 1948), all of whom observed mucous flow in the nose and various sinuses.

The experiments of greatest interest in the present connection are those concerning the trachea. Hach (1925) performed tracheotomy on anaesthetized dogs and then fixed a bronchiole 1 to  $1\frac{1}{2}$  mm in diameter to the parietal pleura. Into this bronchus he dropped a fuscine-stained suspension of lycopodium powder. The anaesthesia was terminated and samples of the tracheal secretion were collected at five-minute intervals through the tracheotomy opening onto a swab. As soon as a trace of the dye indicator appeared the dog was killed. The trachea and lung were then opened and the distance traversed by the dye measured. In this way the rate of mucous flow was calculated and was stated to be 4.6 mm per minute.

A similar technique was employed by Barclay and Franklin (1937), whose experiments were mentioned on page 13. The distance from the lung through the trachea to the larynx (8 cm in the lung and 4 cm in the trachea) took 14 minutes in decerebrate cats. No more detailed information was given of the rate of mucous flow.

Measurements of the transportation of mucus *in vivo* were also made by Florey, Carleton and Wells (1932) and Gordonoff and Mauderli (1936). The former writers made a longitudinal incision into the trachea of cats and through a dissection microscope observed the speed of motion of graphite ink. Only one figure was reported, viz. 35 mm per minute. Gordonoff and Mauderli worked with guinea pigs. Through the lowest of three tracheal incisions the animals were allowed to breathe heated but unmoistened air. Into the middle incision was placed animal charcoal or particles of cork, which acted as transportation indicators. The time taken by these substances to reach the upper opening was recorded. With this method two or three measurements could be made. Thereafter the tracheal membrane became covered with a blanket of viscous mucus, against which the cilia beat apparently without effect. The speed of motion of the animal charcoal was 2.7 to 5 mm per minute.

### The rate of ciliary beat in healthy animals

The rarity of determinations of ciliary beat frequency in living mammals is mainly ascribable to the great technical difficulties involved.

*Lower animals* like mussels, on the other hand, have frequently been studied. Such experiments can be carried out *in vivo* and major problems concerning the maintenance of good physiologic conditions do not arise. All that is required is a chamber flushed through with water of a suitable temperature. The gill cilia or flagella of mussels are relatively long. In many species their beat is not particularly rapid, and they are not packed on the cells in the same way as the cilia of higher types such as mammals. All these factors make the observation of ciliary movement considerably simpler in molluscs than in mammals, and also render possible the study of single ciliary cycles.

The rate of ciliary beat has been estimated with the naked eye, by cinematographic recording and by stroboscopy. One or other of the first two methods was employed by Lucas (1932, 1933 b), Lucas and Douglas (1935), and Lowdnes (1941). Stroboscopic determination of ciliary beat frequency was made by Martius (1884), Hammond (1935) and Bülbring, Burn and Shelley (1953). Filming plus stroboscopy was used by Gray (1930) and Jennison and Bunker (1934).

With the exception of Lowdnes' (1941) study the frequency of photographic exposure was low—as a rule about 30 per second. Lowdnes reported exposures up to a speed of 1/50,000 second. Unfortunately the construction of this apparatus was not described in detail. As the rate of ciliary beat in the lower animals studied generally was much less than that in the respiratory tract of mammals, the results of these earlier experiments are of limited interest for present purposes and will not be further reviewed.

Investigations of the rate of ciliary beat in *mammals* have to date been reported only by Proetz (1933, 1934), and Frenckner and Richtnér (1939).

Determination of the ciliary beat frequency in the mammalian respiratory tract involves many problems. Thus Proetz (1953) pointed out, in describing an apparatus for studying the ciliary activity in living rabbits, that "although such an examination

does not lie without the realm of possibility, the difficulties are enormous as will be seen from the description of the rabbit experiments which follows". The rate of ciliary beat is relatively rapid, it is difficult to obtain acceptable physiologic conditions during the experiments, and the cilia are short and covered with a layer of mucus.

Without being able to count the number of beats per unit of time, Proetz (1933, 1934) succeeded in making a motion picture record of the ciliary activity and wave movement in the mucous membrane of living rabbits. He exposed the maxillary sinus, which was kept moist with physiologic saline. The camera, which had a frequency of about 30 exposures per second, was mounted directly onto a microscope with vertical illumination.

Freneckner and Richtnér (1939, 1940), with an apparatus of their own devising, observed the ciliary activity in *inter alia* human subjects, chiefly in the nasopharynx, nasal conchae and on excised nasal polyps. They recorded a normal range of 160 to 250 beats per minute with an error of 10 to 30 beats.

#### The mechanism of ciliary movement

Many experiments have been performed with the object of elucidating the mechanism of ciliary movement, but the problem is still unsolved. A good survey of the various theories of this mechanism and allied questions was given by Schäfer (1891), Gray (1928) and Heilbrunn (1952).

By and large there are two theories of ciliary movement mechanism. One, advanced by Schäfer (1891), considers that the cilia perform passive movements. According to this writer ciliary motion is brought about by rhythmic pulsation of hyaloplasm in and out of the hollow cilia. Heidenhain (1911), on the other hand, believed the cilia to be active and consist of "a central elastic core with contractile surface", movement being produced by chemical energy transformed into kinetic energy.

As regards a possible centre of movement, it has not been determined with any substantial certainty if this is situated in some special part of the cilium, e.g. the basal corpuscle. Prosser, Brown, Bishop, Jahn and Wulff (1950) maintained that "studies on the progressive anesthetization of the ciliary mechanism have shown

that in protozoans the first thing to be lost is the power of ciliary reversal. At a later stage metachronic coordination is lost, leaving the cilia beating independently. Last of all, the cilia entirely cease their activity. This observation has led to the hypothesis that ciliary beat, metachronism, and reversal are controlled by three separate mechanisms".

Morphologic separation of the movement centres was demonstrated by Worley (1941). In microdissection of intestinal epithelial cells from *Anodonta catarrhacta* Seo, Worley observed various alterations of ciliary movement, depending on the site of incision into the cell. The main findings were that as successive incisions approached the basal corpuscle the normal metachronic beat of the cilia became first synchronous, then increasingly incoordinated and finally, when the incision touched the corpuscle, ceased. From these findings Worley concluded that the ciliary beat as such and its metachronic coordination are not mutually dependent.

The single cycle of ciliary movement has not been determined in mammals. Such studies, however, have been performed on mussels, etc. by Gray (1930), Evans (1945), and Proetz (1953). Two different phases of the cycle were described, a rapid, forward movement and a slower, backward movement. Gray termed these two phases the "effective stroke" and the "recovery stroke". Jennison and Bunker (1934) studied the time relationship of these two phases in *Mya* and found the velocity of the effective stroke to be 3—3.5 times that of the recovery stroke. They also wrote that "during the effective stroke the cilium is quite rigid throughout most of its course while in the recovery stroke it is limp". The angle through which the cilia moved appeared to range from 90° to 180°.

The neural control of ciliary activity has been investigated by a few writers. Only the experiments of Lucas and Douglas (1935) will be mentioned here. In direct microscopy of living turtles these workers failed to observe any alteration of ciliary activity after *inter alia* electrical stimulation of the vagus nerve or tracheal tissue. Nevertheless they pointed out the possibility of altered mucous flow resulting from neural stimulation, since such stimulation may conceivably give rise to changed composition of the secretion. Lucas and Douglas also remarked that even small foreign

bodies may have a stimulating action on the cilia, for which reason transportation indicators should not be used.

### *Morphologic studies*

#### **Light microscopy of the normal tracheal mucosa**

The main aspects of tracheal morphology are well known, *vide* Maximow and Bloom (1952), Ham (1953), de Roberties (1954), etc. To recapitulate briefly: The tracheal lining is a multilayered (pseudostratified) columnar, ciliated epithelium. However, in mouse and rat, the epithelium is composed of simple columnar cells without any tendency to stratification (Frankenhaeuser 1879). Its cells are mainly of two types, ciliary cells and goblet cells. In addition there are basal and intermediate cells. The ciliary, goblet and basal cells rest on a clearly distinguishable basement membrane. Under this is the submucosa with collagen fibrils, elastin, glands, blood vessels and muscle fibres. Here and there in the epithelial layer may also be seen ducts leading from the glands in the submucosa.

The *ciliated cells* which, according to Patzelt (1924), may be observed in the upper portion of the trachea of the human embryo as early as the eleventh week, are considerably more numerous than the goblet cells. They are present from the base of the epiglottis throughout the trachea and out into the smaller bronchi, and are provided with projections—cilia—which appear homogeneous when examined with the light microscope. Just within the cell boundary is a bulb-shaped swelling of the cilium, the basal corpuscle. From this basal corpuscle arise in certain lower animals small fibres which fuse and form the ciliary cone. In mammals, however, these “ciliary rootlets” are not discernible on light microscopy. The number of cilia per cell has ranged in various reports from 8.5 on a cell diameter of 6.2 microns (Lucas, in Cowdry: *Special Cytology* 1932) to 25 to 35 per square micron (Engström and Wersäll, 1952). The length of human tracheal cilia was stated by these writers to be 6 to 7 microns. The mitochondria of the ciliary cells seem to be most abundant apically in the cell, i.e. near the basal corpuscle, although they can also be observed lateral to and under the nucleus.

The question of connections between the cytoplasmic elements and the cilia or basal corpuscles was discussed by such writers as Watanabe (1924), Kindred (1927), and Carter (1928) (cited by Lucas in Cowdry: *Special Cytology*, 1932).

*Goblet cells*, as mentioned above, are the second main type of cell in the tracheal epithelium. They have a secretory function. Together with the submucous glands they secrete mucus into the lumen of the trachea.

Proceeding from observations that the frequency of ciliary motion may be apparently unaltered although the flow of mucus has ceased, Lucas and Douglas (1934) assumed the mucous blanket to be composed of two strata, an upper, highly viscous, resting on the tips of the cilia and a lower, less viscous, surrounding the cilia. Concerning staining of the secretion these writers stated: "Numerous fixed and stained microscopic sections showed that only the tips of the cilia touch the mucus. The outer layer stains with mucicarmine and other stains for mucus, but the underlying fluid layer is uncoloured by commonly used dyes."

Little is known of the *basal and intermediate cells*. The opinion has been for years that there is a progressive development of ciliated and goblet cells from basal cells *via* intermediate cells (Drasch 1879, 1881, Bockendahl 1885).

#### **Electron microscopy of the normal tracheal mucosa**

Of the investigations dealing with the ultrastructure of flagella and the cilia of lower animals may be mentioned those of Schmitt (1939,) Schmitt, Hall and Jakus (1943), Jakus and Hall (1946) and Foster, Baylor, Meinkoth and Clark (1947). For present purposes, however, most interest attaches to the cilia of the respiratory tract.

Ultrastructural studies of these cilia are few. They include the reports of Engström (1951), Engström and Wersäll (1952), and Fawcett and Porter (1954).

Engström (1951) studied the respiratory-tract cilia in different forms of preparations, although not with sectioning, from such higher mammals as dogs and guinea pigs. He found that each cilium had a basal corpuscle approximately 0.5 microns in length and 0.2 to 0.3 microns in diameter. From this corpuscle a rootlet emanated in some animal species but could not be distinguished in others.

The cilium itself, according to Engström, was contained in a thin sheath and consisted of 9 to 11 fibrils. The fibrils were thought to be composed of contractile elements.

Engström and Wersäll (1952) demonstrated in cross sections of human cilia two central fibrils and around them a ring of nine fibrils, all contained in a thin membrane.

An extensive investigation of the cilia in various animals, including mammals, was carried out by Fawcett and Porter (1954). These writers studied cilia from the oviduct of the mouse and from human Fallopian tubes. They also found nine lateral and two central filaments. The peripheral filaments were described as double strands continuing down into the basal corpuscle. In cross sections near the cell surface the nine paired fibrils were no longer distinct, but seemed to fuse to a cylindrical formation. The central pair of fibrils did not continue into the basal corpuscle. Instead there was a central cavity extending from the corpuscle a short distance into the proximal end of the cilium.

The structure of cilia from human Fallopian tubes was not considered to differ significantly from that described for the cilia of the mouse and other animal forms. Ciliary rootlets could not be detected with certainty.

No further electron microscopic studies seem to have been performed which are of interest for the present investigation.

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Many writers have stressed the great importance of normal mucous flow and ciliary activity in the respiratory tract. This was illustrated by functional and morphologic studies of various regions of the respiratory mucosa in conditions such as sinusitis and chronic bronchitis. These affections are characterized by stagnation of the secretions and more or less pronounced morphologic changes in the ciliated epithelium. Thus, in order to describe with any degree of completeness the reaction of the mucosa to various agents, it is necessary to perform both functional and morphologic studies of this membrane.

The functions of most interest are the mucous flow and the

ciliary activity. As many workers have pointed out, such determinations should be carried out in living animals. Otherwise the findings may be of limited value.

The *rate of mucous flow* in the respiratory tract has been measured in several animal species. In most cases, however, the experimental conditions were such that the reported rates must be considered incompletely reliable. This seems to be indicated by the wide discrepancies sometimes apparent in these rates.

The main reason for the lack of conformity probably was that inadequate consideration was given to important physiologic factors such as the temperature of the tissue and the humidity of the atmosphere. Further, the secretory propulsion was investigated with various types of flow indicators placed on the mucosa, which in most cases was moistened with some type of saline solution. Several writers have stated that neither of these two procedures can be considered physiologic. The indicator particles may conceivably act on the mucosa chemically as well as mechanically. Saline solutions may influence mucous flow both by altering the composition of the secretion and by an action on the ciliary activity of the ions contained in the solution.

Because of the technical difficulties in determining the *rate of ciliary beat* in higher animals, the majority of the relatively few reports on this subject derive from experiments performed on molluscs, unicellular organisms, etc. The cilia of such animals are large and beat with fairly low frequency.

The methods earlier used for measuring the rate of ciliary beat were cinematographic recording and stroboscopy. In occasional experiments it was possible to calculate the beats from naked-eye observations. The highest filming frequency hitherto used for this purpose probably was 30 exposures per second. In the present writer's opinion this rate is inadequate for determining rates of more than 8 to 10 beats per second.

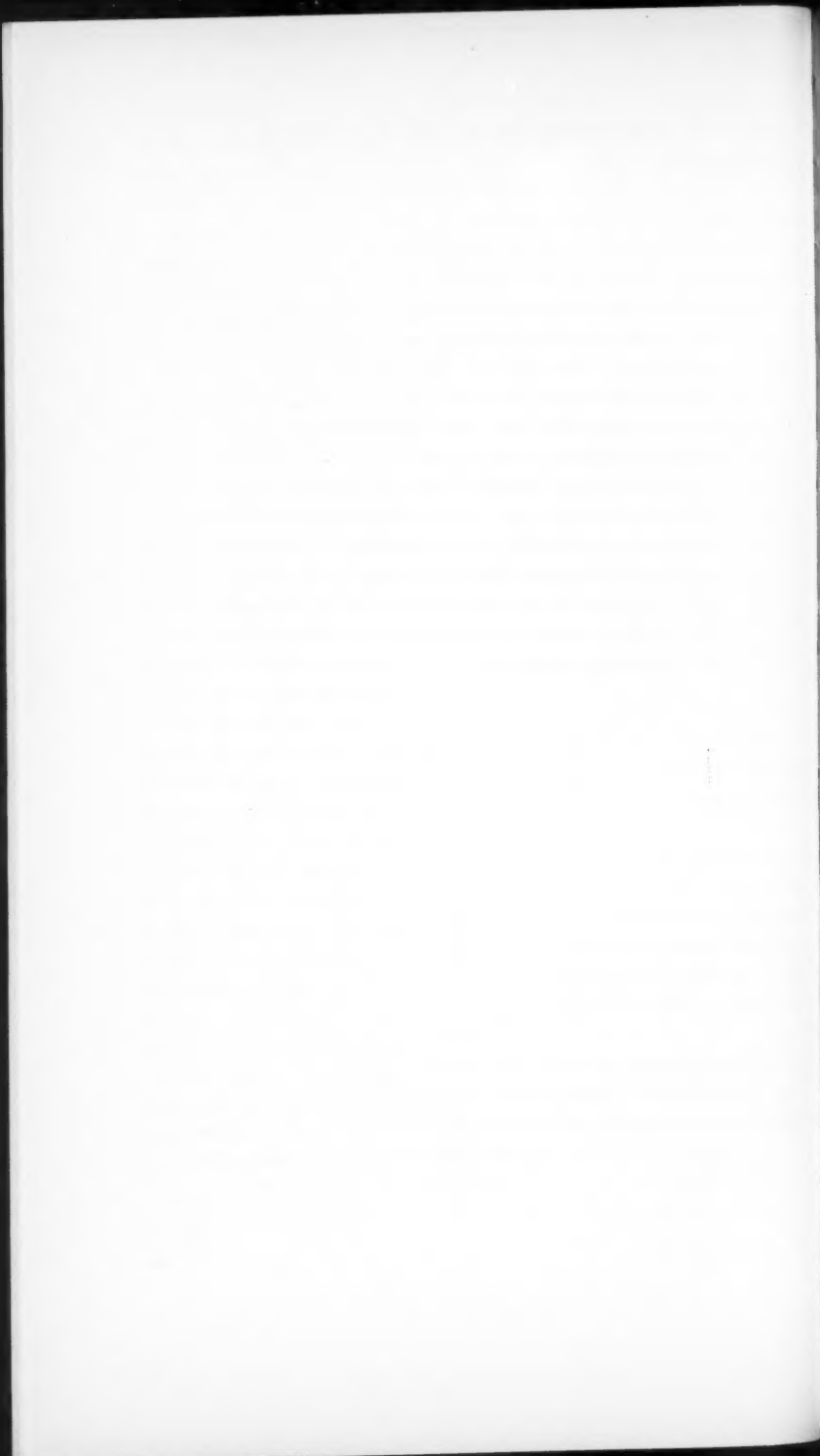
Stroboscopy, according to the literature, has from a technical point of view several advantages in comparison with motion picture filming. The results are immediately available and in the long run stroboscopy is cheaper than filming.

## STUDIES OF FUNCTION

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The importance of simultaneous determination of mucous flow and ciliary activity for comprehension of the tracheal epithelial function was stressed in the General Introduction. The literature cited indicated that such determinations should preferably be made *in vivo*.

No satisfactory method, however, was described for studies of this type. It was therefore necessary to devise a technique for measuring *in vivo* the rates of mucous propulsion and ciliary beat with simultaneous control of certain physiologic factors. These factors were not adequately considered in previous experiments.



## II. Experimental conditions

The main principles of the method, earlier described by Dalhamn in a preliminary report (1955 a), were as follows.

Rats with the trachea slit open were placed in a moist chamber on which was mounted a microscope with vertical light. The *rate of mucous flow* was determined by registering the time taken for shed cells, etc. in the mucus to traverse a certain distance. The *rate of ciliary beat* was measured by making a motion picture record of the ciliary movement as seen in the reflections from the mucosa of the vertical light of the microscope. On subsequent projection of the film the number of beats per unit of time could be computed. Attempts were also made to determine the rate of ciliary beat by stroboscopy. The *phases of a single ciliary beat* were studied by filming the movement of the cilia with a high frequency of exposure. Photomicrographs of the individual film frames then showed the phases of the ciliary beat.

### *Apparatus*

The *moist chamber* (Figure 1) consisted of a glass case with a metal frame. Its outer measurements were: length 60 cm, height 25 cm and width 30 cm. On one short side and on one long side holes 12 cm in diameter were cut to permit working with the hands inside the chamber. These holes were provided with plastic sleeves to prevent entry of air into the chamber. The lid of the chamber was made of plexiglass and was sealed tightly to the walls with rubber packing. Air-tight apertures were made in the lid for an immersion heater, a contact thermometer, stirring devices for water and air, a contacting member for a thermo-electric temperature indicator and a water filter.

The long walls of the chamber were isolated with tinfoil paper and pasteboard in order as far as possible to prevent loss of heat.

The chamber was heated with a 300 W immersion heater placed in a water bath with a capacity of 4 litres. The heater was connected to a contact thermometer with a relay. In order to obtain the greatest possible homogeneity of temperature in the water bath and in the air of the chamber the water was stirred with a mechanical propeller. Above this another propeller on the same shaft brought about air circulation (Figure 2). The air temperature of the chamber was adjustable with the aid of the above-mentioned contact thermometer. Measurements made without animals in the chamber showed that its temperature could be kept constant within an extreme range of  $0.3^{\circ}$  of the desired  $34^{\circ}$  C.

Rapid registration of the chamber temperature was made possible by a *thermoelectric element* (Electric Universal Thermometer, Type

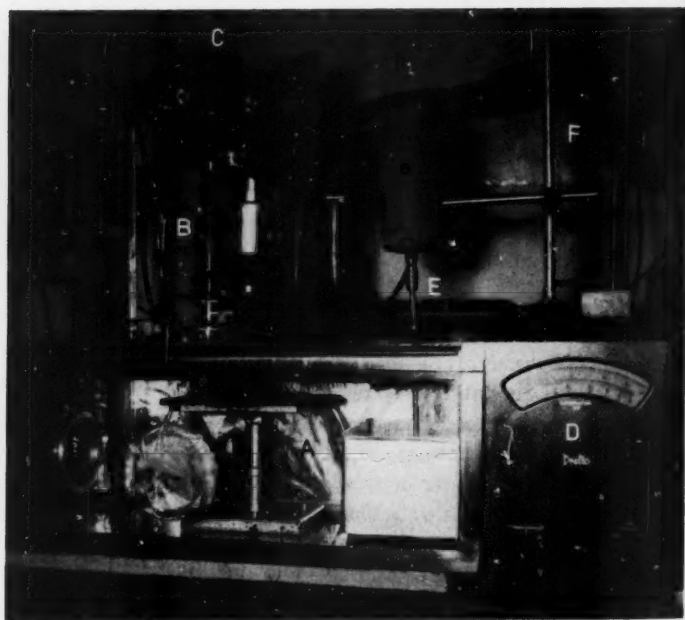


Figure 1. A. Moist chamber. B. Microscope. C. Bell and Howell camera. D. Stroboscope. E. Stroboscope-microscope connection. F. Fan for cooling.

TE 3) connected to the chamber *via* a contacting member. A special contacting member permitted simultaneous readings of the rectal temperature of the experimental animal. The range of the temperature indicator was from  $+16^{\circ}$  to  $+46^{\circ}$  C, divided into tenths of a degree. According to the makers the accuracy of the readings was  $\pm 0.16^{\circ}$ . This instrument was checked and, when necessary, adjusted by simultaneous reading against a precision mercury thermometer.

The humidity of the chamber was measured with a standard hygrometer and could be kept constant at 96 to 98 per cent relative humidity. In the following this atmosphere is regarded as being saturated with water vapour.

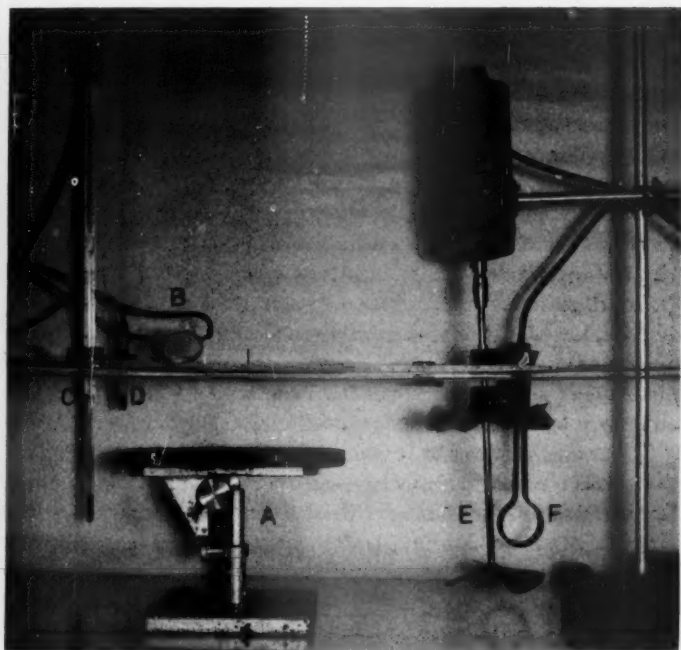


Figure 2. A. Operating table. B. Water filter. C. Contact thermometer. D. Contacting member for thermo-electric temperature indicator. E. Propellers for stirring water and air. F. Immersion heater.

The *microscope* used (Leitz "Ultropak") was constructed for vertical light. This was the only possible illumination for the experiments in question. The objective was a  $6.5\times$ , aperture 0.18.

In selecting the optical equipment of the microscope two factors had to be taken into consideration. The illumination had to be sufficient for high-speed cinematographic recording. The magnifying power of the objective had to be great enough to yield clear observation of the ciliary light reflections and at the same time permit relatively considerable depth of field. The objective was chosen with these requirements in mind.

With the objective U-O  $1.5\times$  the working distance is 110 mm, with U-O  $3.8\times$  it is 33 mm, with U-O  $6.5\times$ , 16.7 mm and with U-O  $11\times$ , 5.8 mm. Only the last two were suitable as regards strength of illumination. U-O  $11\times$ , with its focal distance of 5.8 mm and 0.25 aperture, was rejected, however, partly because of difficulty in obtaining adequate sharpness in viewing. Thus U-O  $6.5\times$ , aperture 0.18, was found to be the most suitable objective for the purpose in view. The ocular was a standard  $5\times$  with a built-in scale. With a microscope tube of standard length this apparatus would have yielded a magnification of  $32.5\times$ . This degree of magnification was not necessary, however, and by shortening the tube the requisite  $17.1\times$  were obtained. The magnification was calibrated with a stage micrometer.

The *illumination* was of two types, that used when filming with a speed of 128 exposures per second and that used for 500 to 1,000 exposures per second. The former was built into the Ultropak microscope in the usual way. The lamp was a Philips "tonfilm" lamp, type 6019 U, designed for 8 volts and 6 amperes. As the lamp body became very warm it was necessary to supply a cooling device. A fan was therefore mounted directly onto the lamp body. The illumination for filming with 500 or more frames per second consisted of an ignition unit and a concentrated-arc lamp (Western Union Telegraph Co. C.A.L., type B-100). The illuminating area of this lamp is concentrated to a surface about one mm<sup>2</sup> (diameter of light source 1.5 mm). The luminance of the light source is 3,900 stilb. The power of the lamp is 100 W. It was mounted on the same framework as the "tonfilm" lamp, but with augmented cooling. The lighting for stroboscopy is described on page 30.

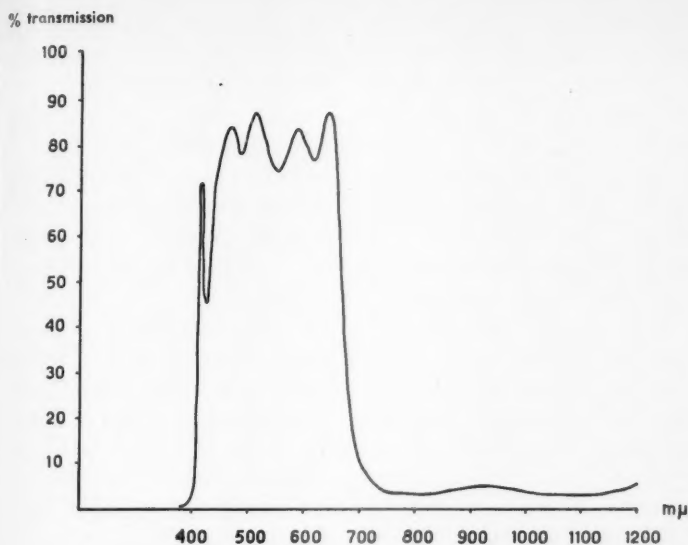


Figure 3. Heat transmission at different wave-lengths of the filter employed.

To prevent heat from the microscope illumination raising the temperature of the tissue under focus *filters* were used. With the apparatus in focus and the temperature of the chamber at normal  $34^{\circ}\text{C}$  the temperature of the examined tissue rose to about  $40^{\circ}$  if heat-deflecting devices were not employed. Two interference filters (Filtroflex type B) were therefore placed in the beam of the "tonfilm" lamp. Control readings with the apparatus in focus without and with this lamp in use showed that in the latter case the temperature of the illuminated tissue was no more than  $0.2$  to  $0.3^{\circ}\text{C}$  above that with the lamp switched off. The heat transmission curve of these filters is shown in Figure 3.

In order to avoid condensation on the observation area of the chamber lid a water filter was built into the lid in the path of the light beam. The construction of this filter is shown in Figure 2. Through it flowed a stream of water with a temperature as a rule  $0.5^{\circ}\text{C}$  higher than the chamber temperature.

The *cameras* used were a Bell and Howell type 70 S for 16 mm

film, with an exposure speed of 128 per second, and a Kodak Eastman High Speed camera type III for 16 mm film, with a speed of 500 to 4,000 exposures per second. For the Bell and Howell camera Ilford HP 3 negative film was used and for the Kodak specially spooled Kodak Super XX reversible film.

The Bell and Howell camera had a spring motor. A large number of determinations showed its speed of 128 exposures per second to be constant. To ensure maximum uniformity, however, each exposure was made for the full time allowed by the spring of the camera.

The speed of the Kodak camera was not constant. Some acceleration took place throughout the filming process, particularly at high speeds. Exact calibration of the camera speed was not attempted. Nor did this seem necessary, since determination of the rate of ciliary beat was not made with this camera. Calibration, moreover, requires an extremely extensive apparatus.

*Camera-microscope connection* was achieved in the case of the Bell and Howell apparatus with a special camera lens (Reichert 4.4:1, aperture 0.08). By filming a stage micrometer the final magnification on the film was estimated to  $4.9 \times$ . A side ocular for focusing the microscope was not considered necessary. Focusing was performed with the microscope in the usual way and the camera was then very quickly swung in over the ocular. No further adjustment of the microscope focus was required to bring the specimen into the image plane of the camera.

The Kodak camera was adjusted directly over the microscope without additional objective or lens (Figure 4). The magnification with this camera was  $4.3 \times$ . It was necessary to focus directly into the image plane of the Kodak camera.

The *projector* used for all films was a Zeiss model with variable projection speed.

The *stroboscope* (Drello) had an illumination frequency range of 300 to 6,000 per minute. Several types of stroboscope lamp were tested, particularly in regard to lighting power, which had to be relatively great for the proposed experiments. A xenon discharge lamp was selected, with a luminous intensity of about 46,000 candles. The energy engendered per illumination was in the region of 0.5 watt/second. The possibility of accurate strobo-

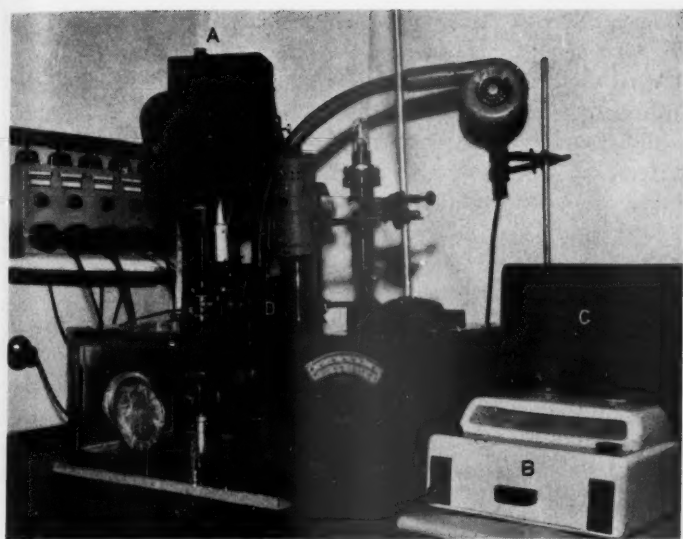


Figure 4. The apparatus with the Kodak-Eastman camera in focus. A. Kodak-Eastman camera. B. Thermo-electric temperature indicator. C. Ignition unit for concentrated-arc lamp. D. Concentrated-arc lamp connected to the microscope.

scopic determinations increases with the brevity of illumination. According to the maker, the stroboscope here used permitted a light duration of approximately  $2 \times 10^{-5}$  second.

The stroboscope lamp was connected to the microscope *via* a condensor lens in such a way that with a simple manoeuvre it was possible to make a motion picture record of ciliary movement in direct association with the stroboscopic measurements. The stroboscope was tested against the known speed of a rotating wheel.

### *Laboratory animals and technique of preparation*

White rats were the animals used. The reasons for this choice were that rats are mammals and their size permits experiments with large groups. Dogs, rabbits and cats entail greater difficulties from

the point of view of accommodation. The trachea of the mouse, on the other hand, is too small for convenient observation.

Whether or not rats in the aspects here studied are comparable with man is a matter for careful consideration. As cilia are very primitive structures and as the respiratory-tract cilia of rats and man perform similar functions, it nevertheless appears not unlikely that a comparison between the two species is warranted.

In the present study, however, comparison was mainly concerned with the mucous flow and ciliary activity in normal *versus* certain pathologic conditions in rats.

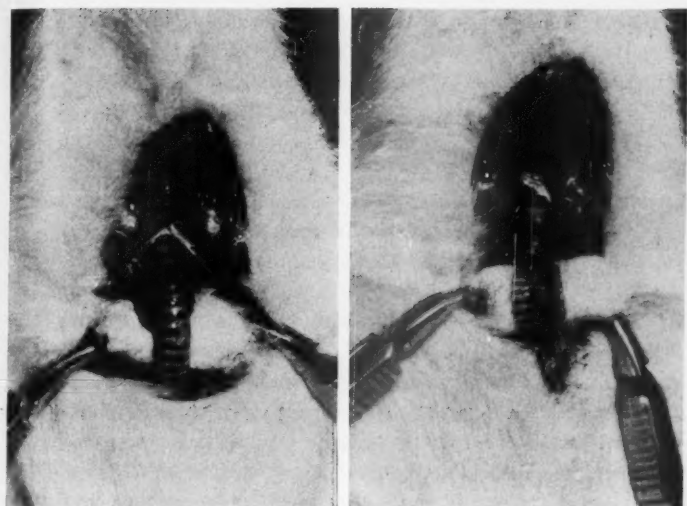
Male rats of Wistar strain were used, with body weight about 300 g, corresponding to an age of about 5½ months. The rats had been reared on the Sherman diet.<sup>1</sup> As infections of the upper respiratory tract could influence the results of the experiments, all rats with body temperature above 37.5° C and all with other signs of such infection were discarded.

*Anaesthesia* was administered by the intraperitoneal route with 1 cc of a 2.4 per cent solution of avertin per 100 g of body weight. Several anaesthetic agents were tested, viz.  $\alpha$ -chloralose, ethylurethane and nembutal. Avertin, however, was found to be preferable for several reasons. In addition to its advantage of rapid action, avertin was easy to administer and did not give rise to complications in the form of pulmonary oedema, the avoidance of which was very important. No investigation was made of the action of avertin on the mucous flow and ciliary activity.

The *preparation* of the rats was as follows: While the animal was anaesthetized the trachea was exposed and the soft tissues between the cartilaginous rings were opened by electrocoagulation. The rings were then cut and the trachea thus opened for microscopic observation (Figure 5). It is unlikely that, except for a purely local effect, the mucous flow and ciliary activity were influenced by this procedure. The electrocoagulation of the intercartilaginous tissue occupied only a few seconds in each rat. It is, therefore, highly improbable that the opposite wall of the lumen, on which the observations were made, was affected by the heat. When the trachea was

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<sup>1</sup> Whole wheat flour 65.8 %, spray-dried whole milk powder 32.9 %, iodized sodium chloride 0.65 %, calcium carbonate 0.65 %.



*Figure 5. The trachea exposed and opened.*

opened the rats were placed in the moist chamber and determinations of mucous flow and ciliary beat were made. There was no risk of drying of the tracheal epithelium in the interval between opening the trachea and placing the rat in the chamber, as this time did not exceed 25 seconds.

### III. Technique of measurements

When the tracheal mucosa of the rats was observed through the microscope three different movements could be distinguished.

First there was seen a smooth stream of secretion containing shed cells, etc., flowing in an oral direction. According to the local conditions in the trachea some degree of light reflection arose. Within this reflection it was possible to observe a wave motion of the secretion. Particularly at the margins of such "high-lights", due to the darker surroundings, another movement could be discerned. This consisted of a very rapid flaring up and fading of points of light. The appearance of this phenomenon was reminiscent of protuberances. In regard to the rate of ciliary beat the very rapid movement was of greatest interest. It is discussible if this movement derives from the cilia themselves or from pulsations in the mucous layer. One way of answering this question would be to place on the mucous layer a fluid with the same refractive index and then use the applied fluid as immersion medium for an immersion objective. If light reflections are still seen, these must derive from the cilia as such. It is extremely difficult, however, to obtain sufficient secretion from the rat trachea for determination of the refractive index. The use of the highly viscous mucus as an immersion medium also presents difficulties. An approach to the problem, however, may be provided by the following considerations.

The cilia are tightly packed on the cells—about 10 per square micron. Consequently the difference in height between two closely-situated cilia will be negligible in a given position of the ciliary movement. If pulsation of the secretion were responsible for the rapid movement of the light reflection, the secretion must follow the height variation of the cilia in their rapid movement. Considering the viscosity of the secretion this would seem improbable.

Pulsations do occur in the secretion, however, and may be clearly seen as waves. These waves are probably caused by the metachronic movement of the cilia. The waves travel relatively slowly and can easily be distinguished from both mucous streaming and ciliary movement. The approximate length of the waves is 20 microns (Figure 6).

As a general rule the determinations were made with the temperature in the chamber approximately  $34^{\circ}\text{C}$  and the rats' rectal temperature  $37^{\circ}\text{C}$ . The atmosphere of the chamber was saturated with water vapour. The time elapsing between opening the trachea and concluding the measurements did not exceed 10 minutes.

The temperature and humidity of the chamber were planned mainly in accordance with the findings from many studies on the trachea of mammals, e.g. Aschenbrandt (1886), and Liljestrand and Sahlstedt (1924). The chamber temperature, according to Perwitzschky (1927), and Ingelstedt (personal communication), should have been about  $36$  to  $37^{\circ}$ . The present writer found, however, that under such conditions the rats' rectal temperature could not be kept constant for a sufficiently long time. As the required stability of rectal temperature was obtained with a chamber temperature of  $34^{\circ}$ , this latter was chosen for the experiments. A rectal temperature of  $37^{\circ}$  was considered normal for the rats.

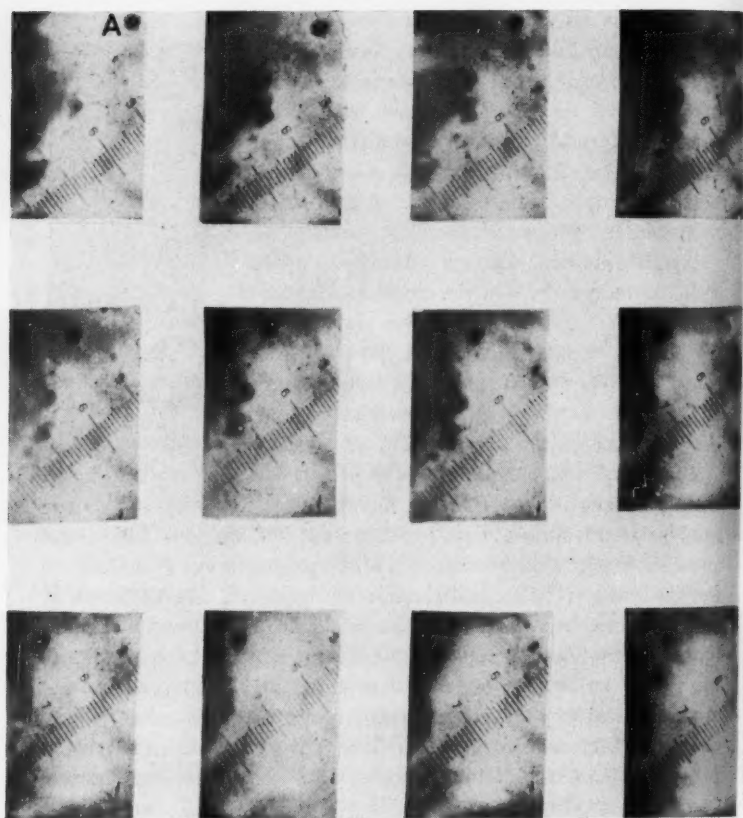
*Special experiments* were performed in which variations in these factors were studied.

### *Determination of the rate of mucous flow*

The rate of mucous flow was estimated by measuring the time taken for certain particles to travel a known distance in the trachea. In each rat three such determinations were made in immediate succession.



Figure 6. The light reflection clearly shows the wave movement in the mucous blanket.

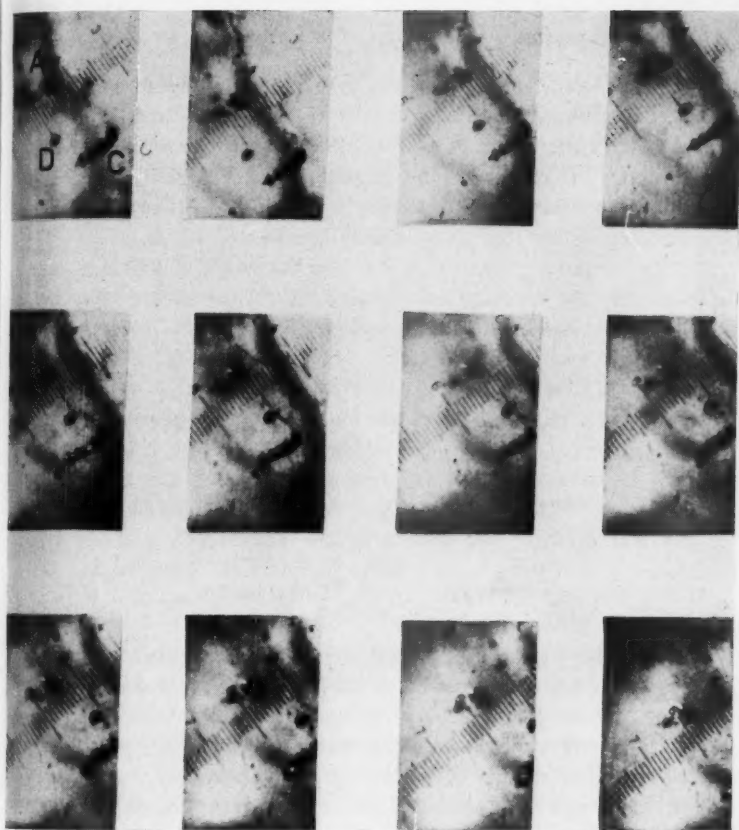


*Figure 7.* The small charcoal particle A moves more rapidly than, and in a short time overtakes the conglomeration of particles, B. The image series comprises every tenth film frame.  
Magnification  $7.2 \times$ .

A scale mounted in the ocular of the microscope showed the distance travelled. Its total length was 1.25 mm, divided on the scale into 50 parts. As flow indicators were used shed cells, etc. in the mucus. These were considered not to produce any local reaction and not to influence mucous flow by reason of their

*Figure*  
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*Figure 8.* Charcoal particles A and B, approximately equal in size, and a larger particle, C, move with similar speed. The progress of particle D is considerably slower. The image series comprises every tenth film frame.

Magnification  $7.2\times$ .

weight. Charcoal particles, etc. were not used as flow indicators as it was found that with such particles the speed of transportation was to a certain extent dependent on their size (Figure 7). Even particles of similar dimensions, however, could have different transportation speeds (Figure 8).

### *Determination of the rate of ciliary beat by filming*

As soon as the speed of mucous flow had been measured the Bell and Howell camera was focused over the microscope and the ciliary movement filmed with a speed of 128 exposures per second. It was important to search for the largest available high-light in the mucosa, as ciliary movement was best seen at the margins of the reflection, against the more weakly illuminated tissue.

At subsequent projection of the film the rate of ciliary beat was calculated. By a ciliary beat is meant here and in the following, when not otherwise stated, a complete movement cycle comprising a forward and a return stroke. The method of calculation was as follows: When the feed wheel of the projector rotated once, ten frames were released. By measuring the number of rotations per minute the number of frames released by the projector in a second could be counted. When the film was projected the time taken to count 50 ciliary beats was measured. Let this time be  $t$  seconds, the rotation speed per minute of the projector  $r$ , and the true frequency of ciliary beat  $x$  beats per minute. The formula will then be  $\frac{128 \cdot 60}{x} = \frac{10 \cdot r \cdot t}{50 \cdot 60}$ , of which  $x = \frac{7,680 \cdot 300}{r \cdot t}$ . This formula was used for all cinematographic records of ciliary beat frequency.

From all the film strips taken for this study, batches of 10 were selected at random. Each batch was then mixed with 3 strips—also randomly selected—on which the rate of ciliary beat, previously determined in another connection, generally was very high or very low, and was presumed to differ from the frequencies registered on the 10 films. On each of these 13 films the rate of ciliary beat was counted thrice, all counts being made in random sequence. The speed of projection was as a rule about 15 frames per second. The object of this procedure was to avoid as far as practicably possible the influence of suggestion. All the film strips were examined in this way by one assistant (Ch. E.).

In order to determine if discrepancies important from a practical viewpoint may arise in counts made by different workers, a test was made with (a) 10 film strips on which the ciliary movement had been calculated by Ch. E. to 1,700 or more beats per minute (all 10 from the experiments shown in table 10), (b) 10 strips selected

at random from the "normal material" (table 4), and (c) 10 strips on which the rate of ciliary beat had been found by Ch. E. to be less than 1,000 per minute (most of these readings are shown in table 11). These 3 film groups, showing high, normal and low beat frequency, were then studied in random sequence by 2 other assistants (G. D. and B. H.). The results are presented in table 1. Between the mean

Table 1. Ciliary Beat Rates Calculated by Different Workers

Name of counter	Rate of ciliary beat		
	Low	Normal	High
G. D. Mean beats/min. ....	775	1,337	2,024
Experimental error(%) ...	11.5	15.3	5.8
Ch. E. Mean beats/min. ....	758	1,380	1,866
Experimental error(%) ...	12.8	8.7	13.7
B. H. Mean beats/min. ....	743	1,478	2,049
Experimental error(%) ...	9.8	13.0	13.9

counts of the different assistants there was no important variation at low and normal frequencies, but at high speeds there was a significant difference ( $0.01^{**} > P > 0.001$ ).

That this difference lacked practical importance in the present study, however, was illustrated by the fact that in the only group of rats which showed accelerated ciliary activity (table 10), this acceleration was recorded not only by the initial counter but also independently by the 2 others.

The possibility that accuracy in counting might be affected by variations in the quality of the films (differences in focusing, thickness of the mucous blanket, appearance of the light reflection, etc.) was also investigated. During the general count of the film strips, Ch. E. classified them into 2 groups as regards clarity—good and less good. In the first group, comprising 117 strips, the experimental error was 12.7 per cent. In the second, comprising 87 strips, the error was 12.9 per cent.

The above similarity may be explained by the fact that, whereas on a "good" film the ciliary beating can be counted the second time the film is projected, considerably longer viewing is necessary before determination can be made on a less clear film. When a favourable site has been detected, however, the ciliary beating can be calculated as easily on the latter as on the former type of film. The

difference between the two, therefore, is mainly a question of the time necessary for counting.

### *Determination of the rate of ciliary beat by stroboscopy*

On page 25 it was mentioned that stroboscopy was used for determination of the ciliary beat frequency. As this method seemed to have several advantages in comparison with cinematographic recording it was considered warrantable to test its usefulness for measuring the rate of ciliary beat in mammals.

The stroboscopic determinations were made with the apparatus described on page 30.

The special difficulties presented by stroboscopic examination are mainly the following.

It is not feasible with either *in vivo* or *in vitro* technique to prepare a specimen on which solitary cilia may be observed.

In accordance with the principles for stroboscopic measurement of motion it was attempted to adjust the stroboscope to a light frequency at which the cilia appear to be stationary. Despite long practice, however, this was not achieved. Some movement always remained. It did not seem possible to calculate how many beats per unit of time this persistent movement represented.

In the stroboscopic determinations it was found to be simplest to begin with illumination frequencies higher than the expected ciliary beat frequency. In this way the following apparent changes of ciliary movement could be observed.

Within the region of the predicted rate of ciliary beat the ciliary movement appeared to decrease, although true immobility was not achieved. At this point it was found that the illumination frequency could be varied by 100 to 200 flashes per minute without apparent alteration in the ciliary movement. If, however, the flash frequency was reduced below this region, the ciliary movement appeared suddenly to undergo great acceleration. This change juncture was chosen by the writer as the reading point in the determinations.

Nine rats were studied under the experimental conditions described on page 35. In each of them the rate of ciliary beat was estimated by stroboscopy in a close succession of readings. The

high-light from which the readings were made was then filmed. It should be mentioned that observation of changes in the ciliary movement with varied stroboscopic flash frequency required a high-light considerably more extensive than that necessary for filming. This was because the stroboscope lamp was much less powerful than the film lamp. The mean rate of ciliary movement as measured by stroboscopy was  $1,099 \pm 14$  beats per minute. The mean motion picture reading was  $1,277 \pm 42$  beats per minute<sup>1</sup>.

The above-described contradictory observations may be explained by the following example. If a circular disc marked with a radial line is rotated at constant speed, and is illuminated with a frequency identical with the number of rotations of the disc per unit of time, the line will appear to be motionless. Each light flash will strike the line in exactly the same phase of the disc's rotation. If the flash frequency is halved the line will still appear stationary. The disc will have rotated twice between each illumination. Doubling of the flash frequency will produce two apparently stationary images to each rotation. Such multiple images will appear with each increase of stroboscopic frequency which constitutes a whole rational fraction of the true rate of rotation. Thus if the relation of the disc rotation to the illumination frequency is 4:3, the disc will move through  $4/3$  rotation between each flash. The eye can register this as three different lines. In such a way the image frequency may be increased until the eye no longer can distinguish the rapid succession of lines. If the number of lines on the disc is not known at the outset, it is not possible to determine the exact number of rotations.

Such multiple images, however, may seem flickering and scanty in comparison with the image seen when the frequency of rotation is synchronous with the illumination frequency. The alteration of flash frequency required to suppress multiple images is very small in comparison with the illumination range within which only one image is seen. When multiple images are registered the slightest shift in the ratio of disc rotation to flash frequency produces the impression of rapid movement of the images.

From these premises the observations in the above-described

<sup>1</sup> This value is not significantly different from the rate of ciliary beat in the "normal material" (1,317 beats per minute).

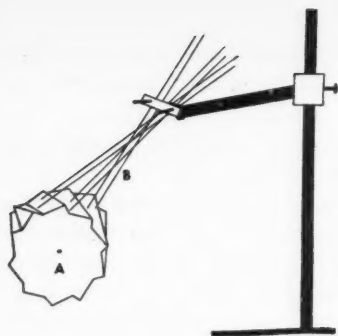


Figure 9. Schematic presentation of experiments illustrating stroboscopic determination of ciliary beat frequency. Over the toothed wheel A trail the wires B. Because of the wheel's obliquely-cut teeth the movement of the wires resembles in rhythm the rapid and slower phases of ciliary movement.

stroboscopic experiments may be interpreted. First there was an apparent slowing of ciliary movement. This slowing persisted within a range of 100 to 200 illuminations per minute. Further reduction of the light frequency resulted in very rapid images. When that stage was reached, therefore, the true rate of ciliary beat had been passed. The change to rapid movement was nevertheless chosen as the point for readings of ciliary beat rate as this point was less difficult to observe than other movement changes.

For further illustration of the stroboscopic determination of ciliary beat frequency the writer constructed an apparatus for model experiments. A toothed wheel against which a number of taut wires was allowed to trail was mounted onto an axle with variable speed. The teeth were obliquely cut and so constructed that the time required for a wire to trail over the short side of a tooth was about one-third of the time required for its passage over the long side (Figure 9). When the wheel was rotated the metal threads, because of the obliquely-cut teeth, were in different phases of movement. In this way a movement was obtained which in rhythm roughly corresponded to normal ciliary movement. Observation with the stroboscope showed the following: If only one wire was used the true frequency of its motion could be determined by stroboscopy. When full synchronization with the illumination frequency was obtained the wire appeared stationary. If all the wires were observed it was extremely difficult to determine accurately the speed of their motion. It was not possible to make all the wires simultaneously appear motionless. Slowing of the vibrations could be achieved, however, at many different illumination frequencies.

It is appropriate to point out that this experiment was carried out under conditions practically ideal from a mechanical viewpoint. Despite this, the true frequency of vibration could not be determined. In observations on mammals such readings must be still more uncertain, partly because the cilia themselves cannot be seen. On the basis of these and the foregoing experimental findings, the writer considers that stroboscopy is not suitable for determining the rate of ciliary beat in animals with respiratory-tract construction similar to that of the white rats here studied.

### *Determination of the phases in a single ciliary beat*

By high-speed filming and subsequent projection of the film, part at least of the two phases of the ciliary beat, the "effective stroke" and the "recovery stroke", could be clearly distinguished for a time sufficient to permit determination of their relationship. As the cilia were observed from above, no reliable conception could be gained of their appearance during a movement cycle. It does not seem feasible, however, to film the cilia of living rats from a lateral view.

Focusing was made with the "tonfilm" lamp with filters in its light beam. Not until the Kodak camera was ready for use were the filters removed and the concentrated-arc lamp switched on. Filming was commenced immediately and took three to four seconds. It was presumed that during this short time the ciliary movement was unaffected by heat from the arc lamp.

When this film was projected a rhythmic flaring up and fading of the light reflection was seen, particularly at its margins. Because the camera frequency was so high (500 to 700 exposures per second) a rapid and a slower phase were clearly distinguishable. More detailed analysis of the single ciliary beat was best made from photomicrographs of the film strip (objective  $6\times$ , ocular  $1\times$ ). A series of such images from a film taken with 700 exposures per second is shown in Figure 10.

A weak point of light is there seen to develop in intensity and size within a few film frames. It then fades and diminishes in size within a relatively large number of frames. Some frames further on, a small point of light again suddenly appears. This sequence of events is repeated with great regularity.

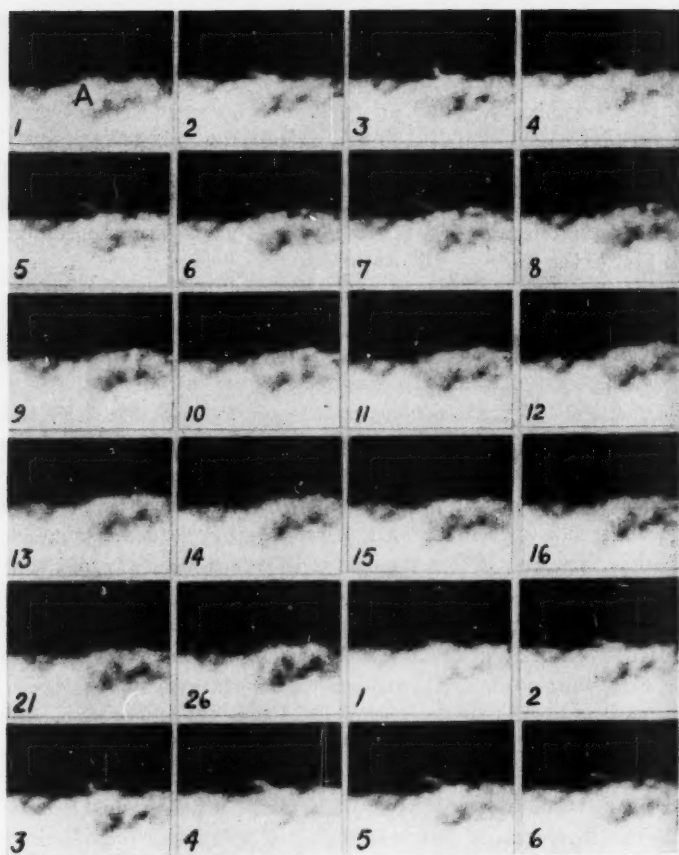


Figure 10. Photomicrographs from a film of the ciliary movement. 700 exposures per second. At A a point of light is discernible, which increases in extent until frame 6. It then diminishes and on frame 21 has quite disappeared. Magnification  $25.8 \times$ .

With the magnification used ( $25.8 \times$ ) the pulsations could not have derived from a single cilium. Instead they must have arisen from groups of cilia. The maximum length of the pulsating highlight was about 0.16 mm and its width 0.02 mm. The visible surface was thus about 0.003 square mm.

Analysis of these images requires discussion of the light beam

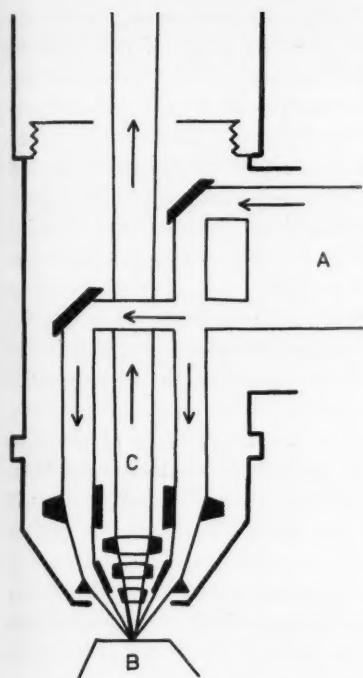
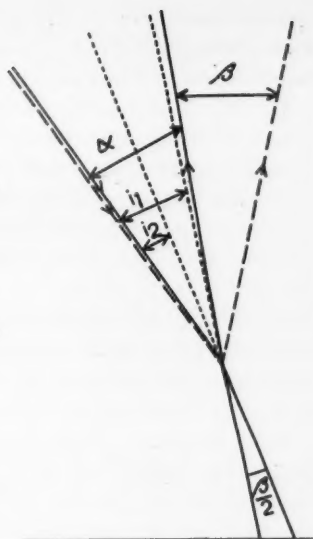


Figure 11.



$$\begin{cases} i_1 = \frac{\alpha + \beta}{2} \\ i_2 = \frac{\alpha}{2} \\ i_1 - i_2 = \frac{\beta}{2} \end{cases}$$

Figure 12.

Figure 11. A. Incident ray. B. Specimen. C. Reflected ray. Figure 12.  $\alpha$  incident ray.  $\beta$  aperture angle of the objective.  $i_1$ ,  $i_2$  angles of incidence of the ray reflected to the outer edges of the aperture angle. These angles of incidence correspond to the position of the cilia in the angle  $\frac{\beta}{2}$ .

and the reflections from the mucosa. The light from the microscope is shown schematically in Figure 11.

The aperture of the microscope's objective was 0.18. If half the aperture angle of the objective is called  $\alpha$  and the refractive index of the air is 1.0 the following equation will be obtained:  $0.18 = \sin \alpha \cdot 1$ ;  $\alpha = 10.4^\circ$ . The aperture angle will thus be approximately  $21^\circ$ . From this it follows that the light reflections appearing in the objective proceeded from a relatively limited area and that a large part of the reflection was never visualized.

It was earlier stated that the light reflections in all probability were produced by the cilia (p. 34). It is now presumed that the light was reflected from the same part of the cilia, whether from the tips or the sides is of no account. Consequently the rays reaching the objective were reflected by a number of cilia at different angles to the cell surface. The angle within which the reflected light could be perceived was  $10.4^\circ$ , i.e. half the aperture angle of the objective (Figure 12). Hence a cilium was visible only for a distance corresponding to  $10.4^\circ$  of its beat. If the light had been reflected from a single cilium the film would have shown a point of light which travelled rapidly over the image field and then disappeared. When a group of cilia traversed the field of vision the adjacent groups also joined in the movement. Because of this the image on the film appeared as a point of light increasing in extent.

It is therefore obvious that, due to the direction of the light beam, the complete ciliary cycle cannot be followed under these circumstances. Parts of the effective and the recovery stroke will then be "blackened out". The darkened part of the cycle corresponds to the film frames in which no movement of the light reflection can be seen or in which an earlier visible reflection has disappeared. The relationship between the visible parts of both beat phases was determined, however, since it is reasonable to regard these parts as equal in the effective and the recovery stroke.

In regard to the film frames in which no movement of the reflection was seen, the most likely explanation was considered to be that the cilia do not have the same maximum angle of deflection in the effective and recovery strokes. If this angle were the same in both phases, a ciliary cycle should comprise two dark periods, i.e. at the extremes of both phases. The dark period, however, occurred only at the end of the recovery stroke and when the cilia turned at the beginning of the effective stroke. In further support of this opinion it is pointed out that the light point began to decrease in size almost immediately, although slowly, after reaching maximum proportions.

\* \* \*

For determination of the mucous flow and ciliary beat rates in the respiratory tract of mammals certain fundamental requirements

were postulated in regard to *apparatus and technique*. These were as follows: The experiments were to be made *in vivo* and preferably on such animals that large series could be used; it should be possible to regulate and control a number of factors which, according to the findings of several writers, may influence mucous flow and ciliary activity. Such factors are air temperature and humidity and tissue temperature. The present writer also considered it desirable that methods for determining mucous flow and ciliary beat rates should be objective and have only a small experimental error.

The moist chamber was found to be suitable for control of these factors, since the temperature could be maintained within narrow limits and the relative humidity at about 100 per cent. Mucous flow and ciliary activity in the rats could be determined at various rectal temperatures.

Determination of the *rate of mucous flow* was made by measuring the time taken for certain particles to travel a known distance. Shed cellular elements, etc. in the mucus were used as indicators of flow. Artificial transportation indicators such as charcoal particles were not employed, as it had been demonstrated that particles of this type, whether unequal or similar in size, could have varying speeds of flow.

This varying speed of artificial transportation indicators may conceivably be attributable to difference in particle size and to local reactions, water absorption, chemical reactions, etc. It is thus clear that estimation of the rate of mucous flow should be made without application of foreign bodies to the mucosa, as not even similarity of size ensures satisfactory results. Conditions are otherwise when the flow indicators consist of shed epithelial cells, etc., which have become fixed in the secretion. Such particles are so small and so scanty in comparison with what may be achieved with artificially applied particles, that they probably do not evoke local reactions of significance for mucous flow. Microscopy shows these cellular elements to flow evenly and with equal speed.

It should also be stated that salt solutions were not used. Nor is application of such fluids advocated. They may conceivably alter the rate of ciliary beat by chemical action and the composition of the mucous secretion by dilution.

The *rate of ciliary beat* was determined by cinematographic recording and projection of the film. It is to be expected that the accuracy of such determinations will increase in proportion to the exposure speed of the camera, i.e. how slow the motion of the cilia appears on the projected film. The writer, therefore, used the standard camera which, in his experience, has the highest constant speed—128 exposures per second.

When a special test was made, in which high, normal and low ciliary beat frequencies were counted by different workers, no significant differences were found except at high beat frequencies (p. 38). It must be kept in mind, however, that the calculated rates cannot unreservedly be accepted as absolutely accurate. When dealing with factors which may accelerate ciliary movement, a modification of the method by increasing the speed of the camera is desirable. This problem, however, was of minor practical importance in the present study, as respiratory irritant gases may *a priori* be expected to have, if anything, a decelerating action on the tracheal cilia. In the only group of rats in the present investigation which showed accelerated ciliary activity, the rising tendency recorded by the initial counter was independently corroborated by two others.

Stroboscopic determination of the rate of ciliary beat was also attempted. Apparent immobility of the cilia, however, was not achieved. The failure to attain this prerequisite for stroboscopic study indicated that the ciliary movement is not strictly regular. This and the anatomic conditions rendered the stroboscopic method of determination inappropriate for the purpose of the present experiments.

The *phases of the single ciliary beat* were recorded with high-speed filming. A speed of 700 exposures per second was successfully employed. Assuming the maximum rate of ciliary beat to be 25 per second, each beat would thus have 28 frames at its disposal. When the film was projected rhythmic pulsations were clearly seen in the entire surface of the light reflection. These pulsations were particularly well seen at the margins of the "high-light", where they contrasted against the darker surroundings.

#### IV. Mucous flow and ciliary activity in the trachea of healthy rats

The apparatus described in Chapter II was designed primarily for *in vivo* studies of mucous flow and ciliary beat rates. The general technique was evolved for experiments on rats, partly because their size is convenient for *in vivo* experiments. The construction of the apparatus, however, does not preclude study of larger animals if such experiments can be made on extirpated tracheal membranes. Certain orienting experiments were therefore undertaken to investigate the degree of conformity between findings in the extirpated trachea and in the trachea *in situ*.

Apart from the feasibility of determining the speed of mucous propulsion and ciliary beat in extirpated tissue, studies on living animals are *a priori* clearly superior, above all when one wishes to measure the immediate effect of certain factors on the function of the tracheal mucosa. On purely theoretic grounds it is realized that changes in, for instance, the temperature of the tissue, or in exogenous factors such as the humidity and temperature of the environmental air cannot be unconditionally presumed to have the same action on extirpated trachea as on trachea *in situ*.

Assessment of disturbances in the function of the tracheal mucosa, whether these are produced by minor alterations in the air temperature or humidity or by damage from gases and vapours, demands a thorough knowledge of the normal functional conditions. With the apparatus here used and strict constancy of experimental conditions it was possible to obtain normal rates of mucous flow and ciliary beat with little variation.

#### *Comparative in vivo and in vitro experiments*

Earlier studies, including those of Lucas (1933 a), Lierle and Moore (1935) and Barclay and Franklin (1937) showed it highly probable that only *in vivo* experiments are of value for determining

mucous flow and ciliary activity. These writers' technique, however, was essentially different from that here employed. For the reasons stated in the introduction to this chapter, therefore, it was decided not to exclude the possibility of reliable results from experiments on extirpated tissue.

This was investigated by *in vivo* and *in vitro* determinations on the same tracheas and by study of the trachea as soon as possible after extirpation.

The *first group* of experiments comprised five rats, in which the rates of mucous flow and ciliary beat were registered first *in vivo* and then *in vitro*. The experimental conditions were otherwise identical with those described on page 35. Thus the air of the chamber was about 34° C with saturated humidity. The rats' rectal temperature was about 37° C. No more than ten minutes elapsed from the time the trachea was opened until the *in vivo* determinations were concluded. The trachea was then excised and fixed on a cork disc. *In vitro* readings of mucous flow and ciliary beat rates were made about seven minutes after removal of the trachea. After a further seven minutes the determinations were repeated.

In these experiments the possibility could not be excluded that the results might be influenced by the fall in tissue temperature from about 37° to 34° C which occurred when the trachea was excised. Nor could this fall be compensated by a rapid rise in the chamber temperature. In order to gain some idea of the implications of such a temperature fall for mucous flow and ciliary activity a *second group* of rats was studied.

This group consisted of three rats. Immediately the trachea was extirpated it was placed in the chamber with an air temperature corresponding to the rats' rectal temperature. The mucous flow was then studied.

In a *third group* (five rats) the trachea was examined as soon as possible after extirpation. The experimental conditions were the same as in the first group. The difference between the groups lay in the length of the interval from opening the trachea to the first *in vitro* determination.

The results from the first and third groups are presented in Tables 2 and 3. They show unanimously that the *speed of*

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Table 2. Mucous Flow and Ciliary Beat Rates In Vivo and In Vitro

Rat No.	In Vivo		In Vitro			
	10 min. after opening trachea		7 min. after removing trachea		14 min. after removing trachea	
	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	5.9	1,154	6.0	1,145	∞	1,245
2	5.5	1,251	20.2	1,308	∞	1,178
3	6.0	1,538	5.9	1,170	∞	1,180
4	5.9	1,111	17.3	1,592	∞	1,185
5	5.7	1,302	5.2	860	∞	1,111
Mean	5.8 (12.9 mm/min.)	1,271	10.9 (6.9 mm/min.)	1,215	∞	1,180
Rectal temperature 36.5°, range 35.8°—37.0°						
Chamber temperature 32.4°, range 31.4°—33.4°						

Table 3. Mucous Flow and Ciliary Beat Rates In Vitro

Rat No.	5 min. after removing trachea		10 min. after removing trachea		20 min. after removing trachea	40 min. after removing trachea
	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.	Rate of ciliary beat beats/min.	Rate of ciliary beat beats/min.
1	10.7	—	∞	1,598	1,689	1,160
2	11.5	—	13.3	1,599	1,089	864
3	21.3	—	∞	—	—	—
4	36.3	—	∞	1,445	1,255	689
5	23.1	—	∞	1,325	1,016	1,070
Mean	20.6 (3.7 mm/min.)	—	∞	1,492	1,262	946
Chamber temperature 32.8°, range 32.0°—34.0°						

mucous flow decreased after extirpation of the trachea. Thus in two of the five rats in the first group the flow was considerably reduced at the end of seven minutes. After a further seven minutes the flow of mucus had ceased in all five rats.

Mucous transportation in the second group, in which the fall in tissue temperature was compensated, ceased not later than six minutes after removal of the trachea.

All five rats in the third group showed a more or less pronounced reduction in the rate of mucous flow five minutes after the trachea was excised. In another five minutes the flow had ceased.

The rate of ciliary beat was registered in groups 1 and 3. In the first group, no definite slowing of the beat frequency was demonstrated ( $P > 0.2$ ). In the third group, slowing of the mean frequency was statistically significant ( $0.05^{**} > P > 0.01$ )<sup>1</sup>.

Both mucous flow and ciliary activity, therefore, underwent deceleration following extirpation of the trachea. When this change first became noticeable in the mucous flow, however, the ciliary beat frequency was not definitely affected.

The speedy reaction of the mucous flow may conceivably have been due to failure of the mucus-producing glands to secrete following interruption of the blood and nerve supplies. The length of the excised trachea was 25 to 30 mm. With a mean flow rate of 13.5 mm per minute the flow should have ceased in about two minutes if no mucous secretion took place after extirpation of the membranes. Diminution or cessation of the flow, however, occurred after 5 to 10 minutes. It would therefore seem probable that the secretion continued for some time after extirpation. The slower reaction of the cilia indicates that their movement mechanism was more resistant than that of the mucous secretion.

Despite their orienting character, therefore, these experiments demonstrated clearly that the applicability of *in vitro* findings is greatly limited. For the problems posed in the present study experiments on extirpated membranes were out of the question.

### *Normal rates of mucous flow and ciliary beat in the rat*

It has been repeatedly pointed out that both mucous flow and ciliary activity are affected by changes in the normal physiology of the tracheal mucosa. A prerequisite for obtaining comparable values, therefore, is that the physiologic environment should be kept intact for as long as possible during the experiments.

Even under constant conditions, however, one must expect to find values which, due to circumstances beyond the control of the

<sup>1</sup> All the significance tests in the present study were performed on the basis of analysis of variance with two or more criteria of classification (*vide* Snedecor 1946).

person conducting the experiments, show a greater or lesser degree of variation. The size of this variation is of course important for the reliability of the normal values. It is also a direct measure of the usefulness of the experimental method. By statistical analysis this variation may be attributed to different factors, such as variations between individual animals, experimental error, etc. From this analysis conclusions may be drawn concerning how great a deviation from the normal value must be in order to be reliably ascribed to some influencing factor. The magnitude of the animals' individual variation around their group mean, moreover, is decisive for the number of laboratory animals required.

The normal material consisted of 37 male rats with a mean body-weight of  $302 \pm 6$  g ( $\sigma = 36$  g).

The general experimental conditions were as described in the chapters II and III.

The temperature of the air in the chamber was about  $34^{\circ}$  C.

The humidity in the chamber was saturated.

The rats' rectal temperature was about  $37^{\circ}$  C.

The time from opening the trachea to concluding the determinations did not exceed 10 minutes.

#### The normal rate of mucous flow

In each rat three determinations were made in immediate succession (Table 4).

These figures express the number of seconds required for a particle to travel 1.25 mm, i.e. the direct reading in the measurement process. The conventional measure of speed—distance/time—is obtained with the formula

$$s = \frac{125 \cdot 60}{t}$$

in which  $s$  = mm per minute and  $t$  = seconds per 1.25 mm. In Figure 13  $s$  is presented as a function of  $t$ . For statistical reasons all primary values are given as seconds/1.25 mm. The mean mucous flow rate for the series is also presented as mm/minute.

A statistical analysis of the primary values with separation of the total variation into different components presupposes normal distribution. This was found to be present when the values were expressed as seconds/1.25 mm ( $g = 0.73 \pm 0.40$ ;  $0.1 > P > 0.05$ ), while significantly skewed distribution was present after transformation to mm/minute ( $g = 1.07 \pm 0.40$ ;  $0.01^{**} > P > 0.001$ ).

Table 4. "Normal" Rats. Basic Data

Rat No.	Weight (g)	Rectal temperature (°C)	Chamber temperature (°C)	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	270	37.0	35.4	5.2; 5.2; 5.2 = 5.2	1,508; 1,464; 1,378 = 1,450
2	304	37.0	35.4	5.2; 5.2; 5.0 = 5.1	1,311; 1,613; 1,270 = 1,398
3	282	37.0	37.0	6.0; 6.0; 6.0 = 6.0	1,473; 2,026; 1,655 = 1,718
4	330	37.0	36.4	5.8; 5.8; 5.8 = 5.8	1,491; 1,060; 1,270 = 1,273
5	370	37.0	32.6	6.0; 6.4; 6.0 = 6.1	1,576; 1,515; 1,419 = 1,503
6	304	37.0	36.0	5.6; 5.6; 5.6 = 5.6	1,498; 1,381; 1,592 = 1,490
7	296	37.0	35.6	4.4; 4.4; 4.4 = 4.4	1,135; 1,312; 1,247 = 1,231
8	336	37.0	34.4	4.8; 5.0; 5.2 = 5.0	1,202; 1,172; 1,052 = 1,142
9	306	37.0	36.0	4.4; 4.0; 4.4 = 4.3	1,417; 1,318; 1,223 = 1,319
10	252	37.0	31.4	5.2; 5.0; 5.6 = 5.3	1,600; 1,495; 1,616 = 1,570
11	262	37.0	35.6	4.8; 4.8; 5.2 = 4.9	938; 977; 931 = 949
12	270	37.0	33.6	5.2; 4.8; 4.6 = 4.9	1,186; 1,200; 1,128 = 1,171
13	252	37.0	36.0	5.6; 5.6; 6.0 = 5.7	1,306; 1,444; 1,133 = 1,294
14	280	37.0	34.4	4.8; 5.0; 5.0 = 4.9	1,463; 1,471; 1,304 = 1,413
15	254	37.0	34.2	6.0; 5.8; 5.8 = 5.9	1,274; 1,238; 1,270 = 1,261
16	270	37.0	34.0	8.6; 8.6; 8.2 = 8.5	992; 1,080; 1,049 = 1,040
17	276	37.0	33.8	8.4; 8.0; 10.0 = 8.8	1,206; 1,180; 1,522 = 1,303
18	308	37.0	34.8	2.8; 3.0; 3.0 = 2.9	1,213; 1,230; 1,322 = 1,255
19	274	37.0	35.0	5.2; 5.6; 5.8 = 5.5	1,706; 1,652; 1,901 = 1,753
20	270	37.0	34.2	4.0; 4.4; 4.2 = 4.2	1,021; 1,118; 1,201 = 1,113
21	344	37.0	32.8	5.2; 4.8; 4.8 = 4.9	1,684; 1,475; 1,495 = 1,551
22	370	37.0	33.4	6.0; 6.0; 6.0 = 6.0	1,145; 1,107; 1,190 = 1,147
23	328	37.2	32.2	6.0; 5.8; 6.0 = 5.9	1,227; 1,347; 1,427 = 1,334
24	318	37.0	33.0	3.8; 3.8; 3.8 = 3.8	1,430; 1,853; 1,479 = 1,587
25	334	37.0	33.8	6.2; 6.0; 5.8 = 6.0	1,524; 1,430; 1,402 = 1,452
26	308	37.2	34.0	5.0; 5.0; 5.0 = 5.0	1,189; 927; 1,215 = 1,110
27	322	36.8	32.8	4.4; 4.4; 4.4 = 4.4	1,172; 1,196; 1,288 = 1,219
28	280	37.0	34.4	6.6; 6.8; 6.0 = 6.5	1,296; 1,135; 1,175 = 1,202
29	250	36.8	36.0	4.2; 4.2; 4.2 = 4.2	1,315; 1,570; 1,407 = 1,431
30	266	37.0	32.8	5.8; 6.0; 5.8 = 5.9	1,157; 1,039; 1,356 = 1,184
31	288	37.0	33.4	6.0; 6.0; 6.0 = 6.0	1,580; 1,433; 1,600 = 1,538
32	268	37.0	31.0	8.4; 8.4; 8.2 = 8.3	1,448; 1,376; 1,306 = 1,377
33	325	36.4	32.4	5.6; 6.0; 6.0 = 5.9	1,061; 1,196; 1,076 = 1,111
34	348	37.0	33.0	5.8; 5.6; 5.6 = 5.7	1,138; 1,314; 1,453 = 1,302
35	338	37.2	35.8	4.0; 4.0; 4.2 = 4.1	1,803; 1,213; 1,178 = 1,398
36	362	37.2	35.0	7.2; 7.2; 7.2 = 7.2	1,166; 944; 884 = 998
37	352	37.2	35.0	6.4; 6.4; 6.4 = 6.4	1,039; 1,075; 1,297 = 1,137
Mean	302	37.0	34.2	5.5 (13.5 mm/min.)	1,317

The normal rate of mucous flow, expressed as seconds/1.25 mm was estimated in the 37 rats as

$$5.55; \sigma = 1.25$$

corresponding to 13.5 mm per minute.

The total standard deviation, 1.25 seconds/1.25 mm, was separated with variance analysis into three components, viz. differences between the animals, differences in the same animal (between the three determinations) and remaining error (*vide* e.g. Snedecor 1946).

The standard deviation between the different rats was found to be only slightly less than the total standard deviation; it was determined as 1.23 seconds/1.25 mm, i.e. 22.3 per cent of the mean.

The standard deviation between the measurements in individual rats is almost to be considered as part of the experimental error, the measurements being made in such close succession (only in exceptional cases did the interval exceed about one minute) that biologically caused changes during that time were not to be expected. The magnitude of this deviation was not greater than the remaining error ( $0.2 > P > 0.05$ ). Nor was there any systematic variation between the first, second and third determinations (respective times 5.53, 5.57 and 5.58 seconds/1.25 mm). Consequently this component was added to the remaining error.

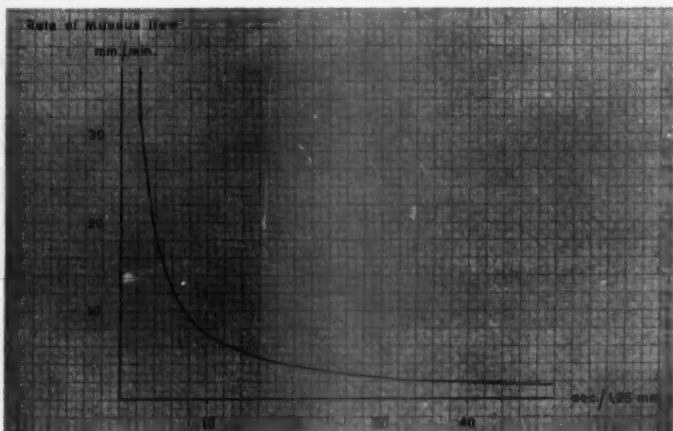


Figure 13. The measurement of mucous transportation (seconds/1.25 mm) transformed into mm/minute.

The experimental error (comprising thus the variation within individual rats and the remaining error) was 0.24 seconds/1.25 mm, or 4.3 per cent of the mean.

The essential cause of the variations in the primary values according to this analysis was differences between the rats. Of considerably smaller dimensions was the cause of deviation termed the experimental error. The difference between the rats, however, must be regarded as quite within the variation range usually present in biologic investigations. The causes of such variations frequently are regarded as random in a wide sense, although they may be produced by definite factors which in their turn show random variation. In some cases, therefore, the variation may be reduced if one of the causal factors can be detected and brought under strict experimental control. It has been stated that the writer as far as possible attempted to maintain constant those factors which according to experience can alter the normal physiologic environment. It was not practicable, however, completely to exclude all variations of the components of this environment. Hence it seemed warranted to investigate if any of the components, despite small deviations, could so have influenced the rate of mucous flow as to have affected the observed readings. The rate of flow was therefore by correlation analysis determined as a function of the air temperature of the chamber, the rats' rectal temperature and their weight. The results are presented in Table 5. They show that no functional connection could be found within the range of these factors. This, however, does not preclude the existence of such a connection if the range of the variables involved is made sufficiently large (see Chapter V).

#### The normal rate of ciliary beat

A cinematographic record of the ciliary beat was made in each of the 37 rats (Table 4). The rate of ciliary beat was calculated according to the method described on page 38.

The figures represent the number of beats per minute. The primary values were analyzed in regard to their statistical distribution. The results show that normal distribution was present ( $g = 0.30 \pm 0.40$ ;  $0.5 > P > 0.40$ ).

Table

Ciliary beat	Mucous flow

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The normal rate of ciliary beat, expressed as beats per minute, was estimated in the 37 rats as

$$1,317; \sigma = 221$$

The total standard deviation, 221 beats per minute, was separated by variance analysis into three components, viz. differences between the animals, experimental error in regard to projection technique (the three counts from the same film strip) and remaining error.<sup>1</sup>

The standard deviation between the rats was 176 beats per minute, or 13.4 per cent of the mean. The standard deviation between the three counts was not significantly different from the remaining error ( $P > 0.2$ ). No systematic difference was present between the respective counts (1,267, 1,342, and 1,323 beats per minute). This component was therefore added to the remaining error. The resultant experimental error was 136 beats per minute, or 10.3 per cent of the mean.

Thus the standard deviation between the rats was not considerably different from the experimental error. Correlation analysis corresponding to that made for the mucous flow rate could therefore scarcely be expected to show any positive reading.

Table 5. *The Influence of Certain Variables on the Rate of Mucous Flow and the Rate of Ciliary Beat*

Variables		Mean	Range	Correlation coefficient	P-value
Mucous flow	Rectal temperature°C.....	36.99 ± 0.02	36.4—37.2	0.135 ± 0.162	0.5 > P > 0.4
	Chamber temperature°C...	34.23 ± 0.24	31.0—37.0	—0.255 ± 0.154	0.2 > P > 0.1
	Weight of rats, g.	301.8 ± 5.9	250—370	—0.035 ± 0.164	0.9 > P > 0.8
Ciliary beat	Rectal temperature°C.....	36.99 ± 0.02	36.4—37.2	—0.019 ± 0.166	1.0 > P > 0.9
	Chamber temperature°C.....	34.23 ± 0.24	31.0—37.0	0.005 ± 0.164	1.0 > P > 0.9
	Weight of rats, g.	301.8 ± 5.9	250—370	—0.098 ± 0.163	0.6 > P > 0.5

<sup>1</sup> Investigation was made of whether or not the frequency of ciliary beat varied in different parts of the observed light reflections. The ciliary movement was studied on three different sites of the same "high-light" on eight film strips. On none of these films, however, was any difference observed between the beat rates at the various sites of the reflection ( $P > 0.2$  in all cases).

It was performed, however, for the sake of consistency (Table 5). The moderate range of the variables in question produced no notable effect on ciliary activity.

### *Phases in the single ciliary beat*

The ciliary beat phases were investigated with the apparatus and the camera speed described on page 28 and 30

Many high-speed cinematographic records were made. On the projected films a rhythmic flaring and fading could be distinguished, particularly at the margins of the mucosal "high-light". Because of the difficulties in obtaining precise focus, the time required for focusing and reloading the camera, etc., accurate determination of the time relationship between the rapid flare and its slower fading was possible only on a few of the films. The most common fault was change in the position of the light reflection while the camera was being finally made ready for operation.

On one film the velocity of the rapid phase was found to be 2.6 times that of the slower phase and on another film 2.7 times (Table 6, Figure 10). Analysis of the films indicated that 500 frames per second is the minimum speed for determination of this ratio. With a presumed ciliary frequency of 25 beats per second, each beat then has 20 film frames at its disposal. If the rapid phase is three times as brief as the slower phase, the former will occupy five of the 20 frames.

Table 6. *Phases of the Single Ciliary Beat*

c. 500 exposures/second		c. 700 exposures/second	
<i>Rapid phase</i> (No. of frames)	<i>Slow phase</i> (No. of frames)	<i>Rapid phase</i> (No. of frames)	<i>Slow phase</i> (No. of frames)
4	11	6	16
4	11	6	17
5	10	6	16
4	10	6	16
4	12	6	16
Mean 4	11	6	16

Thus the motion picture records showed the ciliary movement in the rat trachea to consist of a rapid and a slower phase. The

velocity of the former was calculated on two films to be about three times that of the latter. This ratio tallies well with the 1:3—3.5 found by Jennison and Bunker (1934) in *Mya*.

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The findings from the comparative *in vivo* and *in vitro* experiments showed unanimously that extirpated membranes could not be used for the purposes of the present study. Under certain conditions in which experiments on living animals are technically impossible, for instance in studies on larger animals, the *in vitro* method may be contemplated. This, however, applies only to measurement of the rate of ciliary beat, and if a sufficiently rapid technique of preparation can be evolved.

For determination of the rate of mucous flow and the rate of ciliary beat in healthy rats, the writer's method was found to be satisfactory within the existing variations of the experimental conditions. At this point it is appropriate to reaffirm what has earlier been stated on the subject of ciliary beat frequency (p. 48), as regards variations between the counts of different workers and the writer's opinion of the absolute value of calculated rates of ciliary beat. The variations between the rats, could not be considered remarkable in a biologic material.

The fact that the rate of mucous flow showed a larger interanimal deviation than the rate of ciliary beat indicated that the former function was easily affected by variations in the physiologic environment, while the ciliary activity was more stable and resistant. This was also suggested by the *in vitro* experiments, in which, following extirpation of the trachea, the mucous flow first showed changes. Further evidence that the mechanism of secretion is more easily influenced by variations in the physiologic conditions is presented in later chapters, e.g. in regard to the significance of experimental duration and in the report of the rats subjected to protracted  $\text{SO}_2$  exposure (pp. 60 and 77).

## V. The influence of certain factors on the rate of mucous flow and ciliary beat in healthy rats

The experiments to be presented in this chapter concern the influence of a number of factors on the mucous flow and ciliary beat rates. In the review of the literature it was remarked that in earlier investigations both the general experimental circumstances and the physiologic conditions varied greatly. This dissimilarity was considered to warrant a study of the factors involved.

As shown in chapter II, the writer's apparatus allowed the experiments to be carried out under constant conditions. It also permitted wide variation of these conditions.

The duration of the experiments will first be discussed. An investigation of the influence of humidity and temperature will also be reported, in which consideration was paid to the temperature of the environmental air and of the tissues, and to a combined action of the two temperatures.

### *The significance of experimental duration*

The length of time during which the rats could lie in the moist chamber without showing changes in the mucous flow and ciliary beat rates was of great interest. It provided a measure of how nearly the experimental conditions approached the physiologic ideal. The influence of the experiments' duration on the functions in question was therefore studied.

In six rats the mucous flow and ciliary beat rates were determined 40, 80 and 120 minutes after the trachea was opened. The experimental technique was that described on page 25. The chamber air temperature was about 34° C and the air was saturated with water

Table 7. The Influence of Experimental Duration on Mucous Flow and Ciliary Beat

Rat No.	Length of time in the chamber					
	40 minutes Rectal temp. 37.0° Chamber temp. 34.6°		80 minutes Rectal temp. 37.5° Chamber temp. 34.2°		120 minutes Rectal temp. 37.9° Chamber temp. 35.1°	
	sec./1.25 mm	beats/min.	sec./1.25 mm	beats/min.	sec./1.25 mm	beats/min.
1	4.1	1,398	5.9	1,197	8.7	—
2	6.1	1,447	9.0	1,279	22.0	1,245
3	7.9	1,196	7.1	1,147	∞	1,366
4	5.9	1,381	4.5	1,383	11.3	1,510
5	7.7	998	5.0	1,183	15.3	1,454
6	6.4	1,137	6.0	1,126	6.4	1,103
Mean	6.4 (11.7 mm/min.)	1,260	6.3 (11.9 mm/min.)	1,219	—	1,336

vapour. Both the chamber temperature and the rectal temperature in these experiments showed greater variation than, for instance, in the comparative *in vivo* and *in vitro* studies. The reason was the relatively long duration of the present experiments. The rates of mucous flow and ciliary beat are recorded in Table 7.

The mean rate of mucous flow after 40 minutes (11.7 mm per minute) did not differ significantly from the normal value (13.5 mm per minute). Nor did that measured after 80 minutes (11.9 mm per minute). Forty minutes later, however, i.e. 120 minutes after the trachea was opened, the flow of mucus had ceased in one rat and was considerably retarded in two others.

The mean rate of ciliary beat after 40 minutes was 1,260 beats per minute. The frequencies registered 80 and 120 minutes after the trachea was opened (1,219 and 1,336 beats per minute), showed no statistically significant difference from the initial value ( $P > 0.2$ ).

These experiments, therefore, demonstrated that an experimental duration of at least 80 minutes could be allowed under given circumstances without entailing demonstrable change in the mucous flow or ciliary beat rates. With further prolongation to 120 minutes the mucous flow responded by slowing, while the rate of ciliary beat remained unchanged.

Thus confirmation was provided of the observations in the comparative *in vivo* and *in vitro* experiments and in the normal rats, that the secretory mechanism more easily than the ciliary beat frequency was affected by variations in the physiologic conditions.

### *The influence of the humidity and temperature of the air and of the tissue temperature*

When the animal had been prepared and placed in the moist chamber the humidity and temperature of the air and the rat's rectal temperature were of special importance.

The temperature of the air in the chamber had first to be considered. Under normal circumstances the air inhaled by the rat is satisfactorily moistened and warmed. But when, because of the opened trachea, this became no longer possible, the humidity and temperature of the inhaled air, i.e. the air in the chamber, had to be carefully planned.

Secondly, the tissue temperature, which could be presumed to follow variations in the rectal temperature, had to be taken into account.

The writer will first discuss the literature on these questions. Personal experiments will then be reported.

Many determinations have been made of the *humidity and temperature of inhaled air*. On the other hand, observations concerning the response of the ciliary movement to drying are few. A search of the literature has revealed no study of the effect of varying air temperature on either mucous flow or frequency of ciliary beat.

In regard to the moistening and warming of the inhaled air Aschenbrandt, as early as 1886, stated that in man the air reaching the larynx has a temperature of about 30° C and is practically saturated with water vapour.

Several later studies by and large confirmed Aschenbrandt's findings. Perwitzschky (1927) reported the relative humidity of the air in the human larynx to be 79 per cent. He found that the tracheal mucosa increased this humidity by 16 to 19 per cent, so that when the inhaled air reached the alveoli its moisture content was 95 to

98 per cent. It is particularly interesting to note that mouth breathing appeared to alter these figures. Perwitzschky thus found that with mouth breathing the relative humidity of the laryngeal air was 78 per cent and of the tracheal air 84 per cent. The membranes of the mouth, however, very quickly became dry and could provide moisture for the inhaled air only for a short time. The lowest moisture content was registered in patients breathing through a tracheotomy cannula; in the trachea the relative humidity was no more than 51 per cent.

The temperature of the inhaled air was shown by Perwitzschky to rise in its passage from the nose *via* the larynx to the trachea. In nose-breathing human subjects the temperature of the tracheal air was 35° to 36° C. With tracheotomy respiration it was considerably lower—26° to 28°.

Of other writers who have studied the same problem may be mentioned Gréhan (1864), Schutter (1893), Liljestrand and Sahlstedt (1924), Hellman (1927), Traina (1931), Döderlein (1932), Cone (1933), Seeley (1940), Uddströmer (1940), and Moe (1941). In observations on man or other mammals these writers obtained results similar to Perwitzschky's. The reported tracheal temperatures ranged approximately from 32° to 36° C and the relative humidity of the air in the trachea from 96 to 98 per cent. Traina and Moe also demonstrated that certain variations in the temperature of the inhaled air were only to a very small degree reflected in the nasopharyngeal temperature. Traina varied the atmospheric temperature from 0° to + 25° and Moe from + 6.3 to + 19° C.

Ingelstedt (1952, 1955) evolved an interesting method for determining the humidity and temperature in the trachea of man. In brief, the cricothyroid membrane is punctured with a trocar and cannula. The cannula is then fixed in position and the trocar is replaced by a rapidly registering instrument for measuring humidity and temperature. Ingelstedt thereby demonstrated that the maximum temperature difference in the lumen of the larynx between expired and inspired air during nasal breathing was 5° C. For 70 per cent of the respiratory cycle this difference was at least 4° C.

The relative humidity in the larynx during inspiration was about

93 per cent and during expiration 96 to 97 per cent. Ingelstedt has personally informed the present writer that the tracheal temperature during expiration was about  $36^{\circ}$  to  $37^{\circ}$  C.

The effect of drying on the respiratory mucosa of rabbits was described in detail by Proetz (1933). The procedure employed was simply microscopic observation of a strip of respiratory mucosa exposed to the air. As the membrane dried the ciliary activity decreased and finally ceased. Drying for about 15 minutes produced irreversible damage to the ciliary cells and the ciliary activity was not recommenced by moistening with physiologic saline or Ringer's solution. Proetz stated that "the only natural enemy known to the cilia in their line of function is excessive drying".

In studying the mucous flow and ciliary activity many writers have considered the importance of the *mucosal temperature*.

At this point it is strongly stressed that all the measurements referred to below were made either by observing the cilia without counting the frequency of beat or by estimating the mucous flow with the aid of artificial transportation indicators. Quantitative determination of the ciliary beat and its response to temperature changes does not seem to have been carried out. Subjective assessment of acceleration or slowing of ciliary beat rates and the assumption (which has been expressed) that the speed of mucous flow is directly proportional to the frequency of ciliary beat must be regarded as less than satisfactory.

Changes in the *mucous flow* following variations of the tissue temperature have been observed in several studies.

Hill (1928) examined extirpated tracheal tissue from ox, horse and sheep. The membranes were placed in Ringer's solution. Raising the temperature from  $32^{\circ}$  to  $42^{\circ}$  C resulted in an almost doubled speed of mucous flow. At about  $43^{\circ}$  an unfavourable effect appeared. UEDA (1929) also studied ox trachea in Ringer's solution. The optimal temperature for mucous flow was found to be  $37^{\circ}$  to  $38^{\circ}$  C. A fall to  $15^{\circ}$  resulted in retardation of the flow. Elevation above  $38^{\circ}$  had the same effect.

Gordonoff and Mauderli (1936) observed extirpated frog mucosa and reported the influence of temperature to be very great. According to these writers the optimal temperature lay between  $34^{\circ}$  and  $38^{\circ}$  C.

Other writers have noted changes in the *ciliary activity*. Engel-

mann, as early as 1877, was able with his apparatus for registration of the ciliary movement (Flimmeruhr und Flimmermühle) to demonstrate a 25 per cent increase in ciliary activity when the temperature of extirpated frog membranes was raised from 21° to 41° C. At 45° rapid deceleration began.

The observation of Bergel (1899) and other later writers confirmed Engelmann's findings.

Perhaps the most detailed investigation of thermal effect on ciliary movement was carried out by Gray (1924, 1928). He studied the frontal cilia of *Mytilus edulis*. Under normal circumstances the temperature was found to be the most important factor influencing ciliary and flagellar activity.

Gray's findings in *Mytilus edulis* may be summarized thus: elevation of the temperature from 0° to 33° C resulted in accelerated ciliary movement; at about 37.5° the speed of the beat was reduced and at 40° all movement had ceased; at 45° the cilia passed into "the contracted state of heat rigor" and at 47° they coagulated; between 0° and 28° C the effects of temperature were completely reversible.

Proetz (1934, 1953) studied the influence of temperature on the ciliary activity in excised human mucosa. When the temperature was reduced the ciliary activity diminished and between 7° and 12° C ceased completely. The temperature was then slowly elevated to 30°; at 15° to 19° the cilia were beating "with furious speed", but subsequently showed a gradual return to normal speed. At 43° or more no motion could be seen.

#### **The influence of atmospheric humidity**

The influence of atmospheric humidity on ciliary activity was investigated by the present writer.

For these experiments the humidity of the chamber was varied. The temperature of the air in the chamber was equivalent to room temperature. The rats were prepared in the usual way. The time from opening the trachea to cessation of ciliary motion was registered.

Three degrees of relative humidity were thus tested (~30 per cent, ~50 per cent and ~70 per cent), each on two rats.

At ~30 per cent relative humidity ciliary movement ceased only 3 to 5 minutes after the trachea was opened. At ~50 per cent

Table 8. Mucous Flow and Ciliary Beat Rates with Constant Rectal

Rat No.	Chamber temperature 37° 10 min. after opening trachea Rectal temp. Mean: 36.5°		Chamber temperature 38° 29 min. after opening trachea Rectal temp. Mean: 36.6°	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	7.3	1,166	6.9	1,005
2	5.5	1,194	4.9	1,065
3	8.5	1,061	6.5	1,229
4	4.3	1,146	4.3	1,574
5	8.1	918	6.3	885
Mean	6.7 (11.2 mm/min.)	1,097	5.8 (12.9 mm/min.)	1,152

movement ceased after 8 to 10 minutes. And at ~70 per cent there was no discernible reduction of the ciliary activity when the rats had lain in the chamber for 60 minutes.

In the studies concerning the significance of the experiments' duration, in which the air was saturated with water vapour, no alteration of ciliary beat frequency was registered when the trachea had been open for as long as 120 minutes.

#### The influence of air temperature

In the introduction to this chapter it was pointed out that two writers (Moe and Traina) had shown that reduction of the air temperature exerted only a very slight effect on the nasopharyngeal temperature. Consequently it was not to be expected that such a fall in temperature would influence the mucous flow or ciliary beat rates in the trachea.

The present study of the thermal effects of air on these functions, therefore, was limited to elevation of the chamber temperature. Except for the temperature variations the conditions of the experiments corresponded to the description on page 35.

Two special details, however, will be more closely discussed. When the chamber temperature was raised the rats, because of the saturated atmosphere, were deprived of the possibility of effective heat regulation. This entailed a rise in the rectal temperature.

Temperature 36.8°, range 36.0°—37.6°, and Elevated Chamber Temperature

Rat No.	Chamber temperature 39° 38 min. after opening trachea		Chamber temperature 40° 45 min. after opening trachea		Chamber temperature 41° 53 min. after opening trachea	
	Rectal temp. Mean: 36.8°		Rectal temp. Mean: 36.8°		Rectal temp. Mean: 37.1°	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	7.9	1,074	6.4	1,095	8.5	—
2	6.3	1,215	9.2	1,055	11.3	1,336
3	6.1	1,128	7.9	1,158	7.7	1,075
4	4.1	1,472	4.3	1,308	4.4	1,288
5	4.1	1,363	6.3	1,241	6.4	1,460
Mean	5.7 (13.2 mm/min.)	1,250	6.8 (11.0 mm/min.)	1,171	7.7 (9.7 mm/min.)	1,290

As only the effect of raised air temperature was to be studied, a means had to be evolved whereby the rectal temperature could be kept constant. This was achieved by placing the body of the rat in an ordinary rubber glove, the cuff of which fitted tightly around the upper thorax leaving the head and opened trachea outside. By conducting a jet of air of suitable temperature through the glove the warm air could be removed in amounts depending on the temperature and rate of flow of the cooler air, thus keeping the rectal temperature constant.

It is probable, however, that the temperature of the tracheal tissue could to a certain extent be influenced by raised air temperature, even when the rectal temperature remained constant. The above-described technique, therefore, did not imply that any changes in mucous flow or ciliary activity were caused solely by the altered air temperature. Some role had to be ascribed to an increase in tissue temperature. The latter component, however, was less prominent in the experiments directed at raising the air temperature alone than when the method comprised elevation of the rectal temperature.

The experiments with elevated air temperature were made on five rats. The mucous flow and ciliary beat rates were determined in these rats at chamber temperatures of 37, 38, 39, 40, and 41° C. The results are presented in Table 8.

Table 9. Mucous Flow and Ciliary Beat Rates with Constant Chamber

Rat No.	Rectal temperature 37° 10 min. after opening trachea Chamber temp. Mean: 36.6°		Rectal temperature 38° 19 min. after opening trachea Chamber temp. Mean: 36.5°	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	4.3	1,414	4.4	1,480
2	5.9	1,294	3.3	1,383
3	4.9	1,490	4.5	1,442
4	6.7	—	5.2	—
5	8.4	1,286	5.9	1,247
6	4.5	1,565	4.1	1,136
Mean	5.8 (12.9 mm/min.)	1,410	4.6 (16.3 mm/min.)	1,338

Statistical analysis of the mean speeds of *mucous flow* and *ciliary beat* at the various temperatures showed no significant differences ( $P > 0.2$ ).

#### The influence of tissue temperature

In these experiments the rectal temperature varied. Elevation of this temperature was considered to be of special interest as possibly yielding a conception of the mucous flow and ciliary activity in febrile states.

Two temperatures might then be expected to exert an effect on the functions in question. Elevation of the rectal temperature is followed by heightened temperature in the tracheal mucosa, which in turn raises the temperature of the air traversing the respiratory tract.

The foregoing experiments were concerned with thermal effects on the mucous flow and ciliary activity chiefly from the air temperature. Following the present account of raising the tissue temperature, the results of simultaneous elevation of both these temperatures will be presented.

The rectal temperature was *raised* in two ways. The first was *parenteral administration of a pyrogen* while the chamber temperature was kept constant. The second was *elevation of the chamber temperature*.

The substance employed for parenteral elevation of the rectal temperature was the preparation of *Bacterium alkaligenes* used for

Temperature 36.6°, range 36.0°—37.2°, and Elevated Rectal Temperature

Rat No.	Rectal temperature 39° 39 min. after opening trachea Chamber temp. Mean: 36.8°		Rectal temperature 40° 63 min. after opening trachea Chamb. temp. Mean: 36.7°		Rectal temperature 41° 78 min. after opening trachea Chamb. temp. Mean: 36.6°	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	4.7	1,584	5.6	1,599	6.1	1,676
2	4.3	1,173	6.3	1,178	6.7	1,292
3	4.0	1,547	6.3	1,372	—	—
4	3.3	—	6.0	—	—	—
5	5.6	1,229	10.2	1,393	10.2	1,506
6	4.3	1,043	4.7	1,082	4.7	1,116
Mean	4.4 (17.0 mm/min.)	1,315	6.5 (11.5 mm/min.)	1,325	6.9 (10.9 mm/min.)	1,397

pyretotherapy (State Bacteriological Laboratory, Vaccine No. 1, 1953). 0.6 cc. was injected intramuscularly.

When the temperature of the air in the chamber was elevated the rectal temperature, as previously mentioned, also rose, due to the animal's inability to regulate its body heat in the almost saturated atmosphere of the chamber.

In the experiments with *parenterally elevated rectal temperature* the technique, except for the varying rectal temperature, corresponded to the description on page 35.

Six rats were prepared in the usual way (p. 31). After injection of the pyrogen the rates of mucous flow and ciliary beat were determined at rectal temperatures of 37°, 38°, 39°, and 40° C. The rise in temperature was fairly slow. Only four of the six rats reached 41°.

The data from these experiments are shown in Table 9. The *rate of mucous flow* increased until the rectal temperature had reached 39°. Further temperature elevation resulted in slowing of the mucous flow. Statistical analysis of the mean rates of flow at different temperatures showed significant differences ( $0.01^{**} > P > 0.001$ ).

The *rate of ciliary beat* at different temperatures showed no statistically significant differences ( $P > 0.2$ ).

In the experiments with *elevation of the rectal temperature by means of increased chamber temperature* the readings of mucous

Table 10. Mucous Flow and Ciliary Beat Rates

Rat No.	37° rectal temperature 35.6° chamber temperature, range 32.6°—37.0° 10 min. after opening trachea		38° rectal temperature 37.5° chamber temperature, range 36.0°—38.4° 35 min. after opening trachea	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	5.2	1,450	4.9	1,892
2	5.1	1,398	4.1	1,655
3	6.0	1,718	4.9	1,856
4	5.8	1,273	4.5	1,417
5	6.1	1,503	4.2	1,299
6	5.6	1,490	4.2	1,382
Mean	5.6 (13.4 mm/min.)	1,472	4.5 (16.7 mm/min.)	1,584

flow and ciliary beat rates were made as described above. In six rats these functions were determined at rectal temperatures of 37°, 38°, 39°, 40°, and 41°. The results are presented in Table 10.

Raising of the rectal temperature to about 39° brought about an increase in the *rate of mucous flow*. Higher temperatures entailed deceleration of the flow. Statistical analysis of the mean rates at temperatures of 37°, 38°, 39°, and 40° showed significant differences ( $P < 0.001^{***}$ ). Inclusion of the readings at 41° introduced a greater individual variation between the rats and reduced the significance to a lower level ( $0.05^* > P > 0.01$ ).

The *rate of ciliary beat* at high temperatures was rapid. As it was earlier shown (p. 38) that individual variations in counts made by different assistants were significant at high beat frequencies the writer decided to investigate the practical consequences of such discrepancies by permitting three assistants (G. D., Ch. E. and B. H.) to determine independently the rate of ciliary beat in the experiments presented in table 10. All three determinations showed increasing ciliary activity with rising temperature. According to variance analysis, however, the increasing tendency of the means was statistically demonstrated only in the results obtained by B. H. (B. H.,  $0.05^* > P > 0.01$ , G. D. and Ch. E.,  $P > 0.2$ ).

In the above-described experiments with elevation of the rats' rectal temperature, mucous flow and ciliary beat showed some

## with Elevated Chamber and Rectal Temperatures

Rat No.	39° rectal temperature 38.5° chamber temperature, range 37.8°—40.0° 44 min. after opening trachea		40° rectal temperature 39.6° chamber temperature, range 38.6°—40.8° 52 min. after opening trachea		41° rectal temperature 40.6° chamber temperature, range 39.4°—42.0° 61 min. after opening trachea	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	4.8	1,798	6.9	1,872	9.6	2,065
2	4.7	1,555	5.9	1,858	6.5	1,740
3	4.5	1,559	5.2	1,623	6.7	1,968
4	5.2	1,307	5.9	1,300	7.3	1,517
5	4.0	2,011	6.8	1,733	26.7	1,465
6	4.0	1,358	8.1	1,309	11.6	1,608
Mean	4.5 (16.7 mm/min.)	1,598	6.5 (11.5 mm/min.)	1,616	11.4 (6.6 mm/min.)	1,727

tendency to acceleration or statistically significant acceleration. At temperatures higher than 39°, however, slowing of the mucous flow took place.

In all probability the rise in temperature was responsible for the acceleration. The subsequent slowing, on the other hand, in addition to being a result of thermal influence, could conceivably also have constituted a *fatigue phenomenon* making itself felt at temperatures higher than normal.

The latter possibility was investigated in three rats as follows.

The rectal temperature was raised to 39° by means of elevated chamber temperature. The mucous flow and ciliary beat rates were then determined in the usual way. With the rectal temperature maintained at this level the rats were kept in the chamber for a period equivalent to the mean time required in the otherwise analogous experiments for the rectal temperature to rise from 39° to 41°, i.e. about 15 minutes. The determinations of mucous flow and ciliary beat were then repeated. In neither function had any difference appeared.

The rectal temperature was *lowered* by reducing the chamber temperature. The mean rectal temperature was 31.2° C and the mean chamber temperature 25.2° when the initial measurements of mucous flow and ciliary beat rates were made. These tem-

Table 11.  
Mucous Flow and Ciliary Beat Rates with Reduced Chamber and Rectal Temperatures

Rat. No.	Rectal temp. 31.2° range 29.2°—33.0° Chamber temp. 25.2° range 22.0°—27.4° 95 min. after opening trachea		Rectal temp. 36.9° range 36.6°—37.4° Chamber temp. 35.5° range 34.0°—36.8° 170 min. after opening trachea	
	Mucous flow sec./1.25 mm	Ciliary beat beats/min.	Mucous flow sec./1.25 mm	Ciliary beat beats/min.
1	8.4	877	5.3	1,161
2	16.3	646	4.7	1,308
3	12.7	645	4.1	1,242
4	10.3	617	6.1	1,148
5	22.5	467	7.6	925
6	12.8	671	4.3	1,344
Mean	13.8 (5.4 mm/min.)	654	5.4 (14.0 mm/min.)	1,188

peratures were then raised to 36.9° and 35.5°, respectively, and further measurements were made. The time taken by the entire experiment considerably exceeded the time during which rats could lie in the chamber without affection of the mucous flow (p.60). Nevertheless, the influence of the above temperature changes on the functions in question seemed clear. Thus the mean rate of mucous flow increased (table 11) from 5.4 to 14.0 mm per minute ( $0.01^{**} > P > 0.001$ ) and the mean frequency of ciliary beat from 654 to 1,188 beats per minute ( $P < 0.001^{***}$ ).

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The findings in regard to the influence of humidity and temperature on mucous flow and ciliary activity confirmed the writer's opinion that variations in these factors preclude constant experimental results. This implies that some reports on relevant problems are of little interest. Such are studies in which the conditions of the experiments were uncontrolled or inadequately controlled. For reproducibility of results it is essential to have experimental conditions which are constant and can be strictly regulated.

The experiments concerning the significance of time for the rates of mucous flow and ciliary beat showed that the rats could lie in

the chamber for at least 80 minutes with these functions unimpaired. Mucous flow subsequently displayed reactions. Ciliary activity, on the other hand, was not demonstrably affected after 120 minutes. Eighty minutes must be considered adequate time for single determinations after, for instance, exposure to gases, etc. The measurements can almost always be made within ten minutes of opening the trachea. It is also possible during the remaining time (70 minutes) to make continuous registration of the reactions of mucous flow and ciliary activity to orally and parenterally administered drugs.

The time studies thus showed that mucous flow was affected earlier than ciliary activity. The reason, as indicated already in this chapter, was that the secretory mechanism reacts more rapidly than the ciliary mechanism to changes in the physiologic environment. The retardation of the mucous flow while the ciliary beat frequency remained unaltered must imply changes in the composition (e.g. the viscosity) or amount of the secretion. It is not difficult to conceive of such changes arising from impaired activity of the autonomically innervated glands.

The physiologic significance of the atmospheric humidity as herein demonstrated, was in complete conformity with earlier findings on the same question. A low relative humidity within a short time brings about drying of the mucosa with resultant arrest of secretory flow. This in turn increases the risk of bacterial infiltration and infections of the mucosa. Such clinical aspects, however, are not within the scope of this study and therefore will not be further discussed.

The findings concerning the influence of different temperatures may be summarized as follows: When only the air temperature was raised, both mucous flow and ciliary activity appeared unaffected. When only the rectal temperature was raised, the speed of mucous flow increased until the temperature reached 38 to 39°C. Further temperature elevation entailed deceleration of this function. No effect on the ciliary activity was demonstrable.

When both the chamber and the rectal temperatures were elevated, the reaction from the mucous flow was similar to that in rectal temperature elevation alone. It was demonstrated that retardation of the mucous flow at temperatures higher than 39° did not constitute a fatigue phenomenon.

A clear tendency to acceleration of the ciliary movement was discernible when the chamber and rectal temperatures were concurrently raised.

Elevation of the rectal temperature by means of increased chamber temperature may from a physiologic aspect correspond to the conditions in fever, in which a rise in body temperature entails heightened temperature of the tracheal air.

It is not hereby inferred, however, that the relationship between the chamber temperature and the rats' rectal temperature in the present experiments was in direct conformity with the temperature relationship in febrile states.

The temperatures had to be achieved within 80 minutes of opening the trachea and the writer elected to make the readings at whole degrees of rectal temperature. The elevation of the chamber temperature, therefore, had to be regulated so that the rectal temperature reached the desired maximum value— $41^{\circ}$ —within these 80 minutes. It is conceivable that the chamber temperature at the various whole degrees of rectal temperature did not correspond to the air temperature normally present in the trachea at the rectal temperatures in question. The experiments, however, were conducted so that a rise of one degree in rectal temperature as a rule was concurrent with a one-degree rise in chamber temperature. Hence it is probable that no major discrepancies existed as compared with the temperature relationship in febrile states. By and large the experiments seemed to indicate that effectivization of mucous flow and ciliary activity occurs in conditions in which the rectal temperature is elevated up to a certain limit.

In this connection it is pointed out that the experiments with elevation of the chamber *or* the rectal temperature, cannot be considered "pure": Elevation of the chamber temperature with constant rectal temperature did not necessarily preclude a rise in the temperature of the tissues—in this case the tracheal mucosa—in direct contact with the air. As it is in this surface region that the cilia exist, they may have been influenced by such a rise in the tissue temperature. In the writer's opinion, however, it is reasonable to regard the main effect on mucous flow and ciliary beat as deriving from the elevated air temperature. Similar arguments may be advanced in regard to the experiments with constant air

temperature and elevated rectal temperature, although here the question is of a possible cooling of the tracheal epithelium.

Simultaneous lowering of the chamber temperature and the rectal temperature entailed deceleration of mucous transportation and ciliary activity. When these temperatures were raised to values approximating normal, both functions responded with significant acceleration. The rates shown, however, cannot be directly compared with, for instance, those in normal rats, as the time which elapsed before even the first flow readings were made exceeded the interval during which the mucous transportation remained unaffected under normal conditions (p. 60). The influence of temperature reduction, however, may be considered clearly demonstrated.

## VI. Functional reactions in the tracheal mucosa after protracted exposure to sulphur dioxide

The effect on the respiratory mucosa of protracted exposure to respiratory irritant gases is particularly important from the aspect of industrial health. Many industrial occupations entail more or less protracted exposure to gases and solvents. Of these substances not a few exert irritant effects primarily on the respiratory tract (Fairhall 1949, Hamilton and Hardy 1949, Elkins 1951, etc.). In a survey of the effects of sulphur dioxide inhalation in man and other mammals Greenwald (1954) wrote: "the evidence regarding chronic intoxication is rather conflicting. However, it appears that occasional, even frequent, exposures to rather high concentrations of sulfur dioxide are not as harmful as continuous (working-day) exposures to lower, apparently more tolerable, concentrations."

From Greenwald's review of the literature it would seem that 10 p.p.m.  $\text{SO}_2$  may be too high a concentration for prolonged exposure and, moreover, that rats probably are relatively resistant to this gas, as compared with mice, guinea pigs and man.

Amdur, Melvin and Drinker (1953) demonstrated convincingly that inhalation of 10 p.p.m.  $\text{SO}_2$  produced changes in the normal respiratory pattern.

Clinical studies of this problem have been carried out by Herzog and Pletscher (1955). With few exceptions these investigations were concerned with single exposure. By means of bronchoscopy and biopsy a number of histologic changes in the mucosa could be observed after very brief but frequently intensive exposure. Concerning the action of protracted exposure to respiratory irritant gases few clinical investigations have been reported.

The unpublished studies of Lundgren<sup>1</sup> should be mentioned, however. Lundgren has carried out bronchoscopic and histologic investigations on men and animals exposed for long periods to low concentrations (10 p.p.m.) of sulphur dioxide. All the animals and many of the human subjects showed chronic bronchitis.

How the mucous flow and the ciliary activity react to pulmonary irritant gases has not previously been investigated. A probable reason was lack of a suitable method for registration of these functions.

With the technique of measuring mucous flow and ciliary beat rates described in Chapters II and III, the writer has studied the effect of prolonged exposure to low concentrations of SO<sub>2</sub>. A preliminary report has been published by Dalhamn (1955 b).

### *Method of exposure*

The rats were exposed to SO<sub>2</sub> with the aid of the apparatus shown in Figure 14. From a cylinder containing approximately 10 per cent SO<sub>2</sub> a capillary manometer designed for 1 to 3 litres per hour was connected to a mixing vessel. Into this vessel was also conducted air *via* a drying tower and a flow meter. The air-SO<sub>2</sub> mixture was then led into an exposure chamber. The total volume of the mixture passing through the chamber was registered. By adjusting the constituents of the mixture a concentration of about 10 p.p.m. SO<sub>2</sub> was obtained for the experiments. The total circulation of the air mixture in the exposure chamber was about 30 litres per minute. The SO<sub>2</sub> concentration was selected with regard to the current maximum allowable concentration value (Ahlmarm and Bäcklund-Larsson 1954). Sampling and analysis of the gas were made with the method described by Jacobs (1953).

### *The influence of sulphur dioxide on the mucous flow and ciliary activity*

In studying mucous flow and ciliary activity in the "exposed" rats great care was taken to achieve experimental conditions

<sup>1</sup> At the Department of Occupational Health, the National Institute of Public Health.

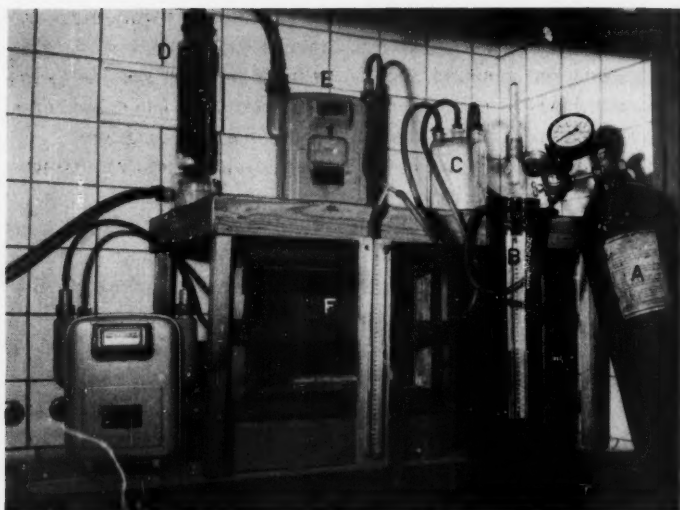


Figure 14. A. Cylinder with circa 10 p.p.m.  $\text{SO}_2$ . B. Capillary manometer. C. Mixing vessel. D. Drying tower. E. Flow meter. F. Exposure chamber.

conforming to those in the experiments on healthy rats. Such conformity permitted any disparity between the healthy rats and those described in the present chapter to be accepted without reservation.

The method of exposure to  $\text{SO}_2$  also entailed exposure to a continuous stream of air. It was therefore discussible if changes could be produced in the tracheal epithelium by the air stream without added  $\text{SO}_2$ . Six rats comprising a special group of "air controls" were therefore exposed for 62 days to the air inflow

Table 12.  $\text{SO}_2$  Content in Air Samples

Day of exposure	p.p.m.
1	10.2
10	13.4
18	10.6
Mean	11.4

alone, but under conditions otherwise identical with those employed in the following groups.

Two groups of nine rats were submitted to *protracted exposure to sulphur dioxide*. In group 1 the rats were exposed for 18 days to a mean  $\text{SO}_2$  concentration of 11.4 p.p.m., and in group 2 for 67 days to 11.5 p.p.m. (Tables 12 and 13). In both groups exposure

Table 13.  $\text{SO}_2$  Content in Air Samples

Day of exposure	p.p.m.
1	11.3
2	12.1
3	14.4
4	13.2
5	10.7
15	10.0
24	10.8
39	12.3
67	10.1
Mean	11.5

was continued for six hours on five days of the week and for three hours on the sixth day. An interval was allowed on Sundays. At the beginning of the observation period the  $\text{SO}_2$  concentration was determined daily in group 2. As the variations were too small for significance in the present connection, however, later analysis was confined to random samples. At the conclusion of exposure each group of rats was divided into two subgroups, one of six and the other of three rats. Immediately the  $\text{SO}_2$  exposure had ceased the six-rat groups were examined in regard to mucous transportation, rate of ciliary beat and morphologic reactions (see Chapter IX). The three-rat groups were not so examined until 33 days after the cessation of exposure. The purpose of these later studies was to observe possible regression of changes which might have arisen in the tracheal mucosa following  $\text{SO}_2$  exposure.

#### "Air controls"

The findings in the "air controls" are presented in Table 14. No statistically demonstrable deviation from the normal values of mucous flow and ciliary activity (5.5 seconds per 1.25 mm,

corresponding to 13.5 mm per minute, and 1,317 beats per minute) was registered in the air controls (5.7 seconds/1.25 mm, corresponding to 13.2 mm/minute, and 1,384 beats/minute). The statistical

Table 14. Exposure to the Air Inflow of the Chamber for 62 Days

Rat No.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	6.2	1,605
2	5.1	1,159
3	5.9	1,346
4	6.1	1,807
5	6.4	1,278
6	4.3	1,108
Mean	5.7 (13.2 mm/min.)	1,384
Rectal temperature 36.7°, range 36.2°—37.0°		
Chamber temperature 34.3°, range 32.6°—35.2°		

analysis showed variations essentially in conformity with the "normal material" (Table 15).

Table 15. Survey of Experimental

	"Normal material"	"Air controls" for 62 days
Mucous flow (mm/min.) . . . . .	13.5	13.2
Mucous flow sec/1.25 mm . . . . .	5.5	5.7
Standard deviation between rats (%) *	22.3	14.3
Experimental error (%) * . . . . .	4.3	3.0
Ciliary beat (beats/min.) . . . . .	1,317	1,384
Standard deviation between rats (%) .	13.4	18.6
Experimental error (%) . . . . .	10.3	10.5
Rectal temp. (°C) . . . . .	37.0	36.7
Range . . . . .	36.4—37.2	36.2—37.0
Chamber temp. (°C) . . . . .	34.2	34.3
Range . . . . .	31.0—37.0	32.6—35.2
No. of Rats . . . . .	37	6
* The statistical analysis was performed on primary values expressed		

# **Eighteen days' exposure to 11.4 p.p.m. sulphur dioxide**

The results in the six rats exposed to a mean SO<sub>2</sub> concentration of 11.4 p.p.m. for 18 days and examined immediately thereafter are presented in Table 16.

Table 16. Exposure to 11.4 p.p.m. SO<sub>2</sub> for 18 days

Rat No.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	∞	1,139
2	7.3	1,045
3	∞	1,048
4	∞	1,160
5	5.3	1,000
6	6.5	1,261
Mean	—	1,109
Rectal temperature 36.9°, range 36.8°—37.0°		
Chamber temperature 33.2°, range 31.0°—36.0°		

A considerably increased amount of mucus was noted as soon as the trachea was opened. The mucosa appeared reddened. These findings are discussed in more detail in Chapter IX. In three of the six rats no mucous transportation was seen. In the other three the rate of flow was within normal limits. The ciliary activity was

## *Results (showing temperature range)*

11.4 p.p.m. SO <sub>2</sub> for 18 days	11.4 p.p.m. SO <sub>2</sub> 18 days, then 33 exposure-free days	11.5 p.p.m. SO <sub>2</sub> for 67 days	11.5 p.p.m. SO <sub>2</sub> 67 days, then 33 exposure-free days
{flow ceased in 3 rats	{flow ceased in 1 rat	{flow ceased in 1 rat	{flow ceased in 1 rat
—	—	—	—
—	—	—	—
1,109	1,008	1,320	1,168
7.5	5.1	13.2	10.5
7.6	11.5	9.2	8.5
36.9	37.0	37.1	37.0
36.8—37.0	36.8—37.2	37.0—37.4	36.6—37.2
33.2	34.1	34.1	34.5
31.0—36.0	31.6—35.4	31.4—36.0	33.4—35.4
6	3	6	3
as sec./1.25 mm.			

clearly observed in all six rats. The mean rate of ciliary beat was 1,109 per minute, which was significantly different from the 1,317 beats per minute in unexposed rats ( $.05^* > P > 0.01$ ).

Of the three rats studied 33 days after the cessation of exposure (Table 17), one showed no measurable transportation of mucus and

Table 17. Exposure to 11.4 p.p.m.  $\text{SO}_2$  for 18 Days, Followed by 33 Exposure-Free Days

Rat No.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	6.0	938
2	20.0	984
3	$\infty$	1,101
Mean	—	1,008
Rectal temperature $37.0^\circ$ , range $36.8^\circ$ — $37.2^\circ$		
Chamber temperature $34.1^\circ$ , range $31.6^\circ$ — $35.4^\circ$		

in another the flow was retarded. The mean rate of ciliary beat—1,008 per minute—differed significantly from the value in unexposed rats ( $0.05^* > P > 0.01$ ).

The statistical analysis showed variation in conformity with the “normal material” (Table 15).

#### Sixty-seven days' exposure to 11.5 p.p.m. sulphur dioxide

The results in the six rats exposed to a mean  $\text{SO}_2$  concentration of 11.5 p.p.m. for 67 days and examined immediately thereafter are presented in Table 18.

Table 18. Exposure to 11.5 p.p.m.  $\text{SO}_2$  for 67 Days

Rat No.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	6.6	1,579
2	8.7	1,189
3	9.9	1,074
4	10.4	1,465
5	34.4	1,238
6	$\infty$	1,376
Mean	—	1,320
Rectal temperature $37.1^\circ$ , range $37.0^\circ$ — $37.4^\circ$		
Chamber temperature $34.1^\circ$ , range $31.4^\circ$ — $36.0^\circ$		

As in the rats exposed to  $\text{SO}_2$  for 18 days, a considerably increased amount of mucus was seen as soon as the trachea was opened. The mucosa appeared reddened. In one of the six rats there was no measurable mucous transportation and in another the flow was greatly retarded. Two of the remaining four rats also showed slowing of the mucous flow, although to a lesser extent. The mean rate of ciliary beat—1,320 per minute—did not differ significantly from the rate in unexposed rats.

In one of the three rats examined 33 days after  $\text{SO}_2$  exposure there was no measurable transportation of mucus and in another the flow was retarded (Table 19). The mean rate of ciliary beat—1,168 per minute—was not significantly different to that in unexposed rats.

Table 19. Exposure to 11.5 p.p.m.  $\text{SO}_2$  for 67 Days,  
Followed by 33 Exposure-Free Days

Rat No.	Rate of mucous flow sec./1.25 mm	Rate of ciliary beat beats/min.
1	6.7	1,114
2	12.4	1,068
3	$\infty$	1,322
Mean	—	1,168
Rectal temperature $37.0^\circ$ , range $36.6^\circ$ — $37.2^\circ$		
Chamber temperature $34.5^\circ$ , range $33.4^\circ$ — $35.4^\circ$		

The statistical analysis showed variation in conformity with the "normal material" (Table 15).

### *The effect of sulphur dioxide exposure on the single ciliary beat*

Retardation of the mucous flow was thus observed in all four groups of rats exposed to  $\text{SO}_2$ . The ciliary beat frequency, on the other hand, was affected in only two groups, in which it was diminished.

Changes in the speed of mucous flow may in general be produced by many factors. The depth of the mucous layer, the secretion's viscosity, the rate of ciliary beat and the relationship between the effective and recovery strokes all play decisive roles in maintaining

the rate of mucous flow. Even if the ciliary beat and other factors are constant, a change in the relationship of the effective and recovery strokes may bring about altered transportation of mucus.

This relationship was studied in rats exposed to  $\text{SO}_2$ . The method was that described in Chapters II and III. Three rats were used. With the procedure reported on p. 77 they were exposed for 48 days to a mean concentration of 10.4 p.p.m.  $\text{SO}_2$  (Table 20).

Table 20.  $\text{SO}_2$  Content in Air Samples

Day of exposure	p.p.m.
1	9.2
3	9.1
19	13.3
21	9.7
27	10.2
48	10.9
Mean	10.4

The ciliary movement in the three rats was filmed with a speed of about 500 exposures per second immediately after the  $\text{SO}_2$  treatment had ceased.

All three motion picture records showed a rhythmic pulsation of parts of the light reflection. Only one film, however (Figure 15) was suitable for detailed study. Five ciliary beats from this film gave the following values for the ratio between the time taken for a definite point of light to reach its maximum and the time occupied by its recession (Table 21).

Table 21. Phases of the Single Ciliary Beat

c. 500 exposures/second	
Rapid phase (No. of frames)	Slow phase (No. of frames)
4	13
4	13
4	12
4	12
4	12
Mean 4	12

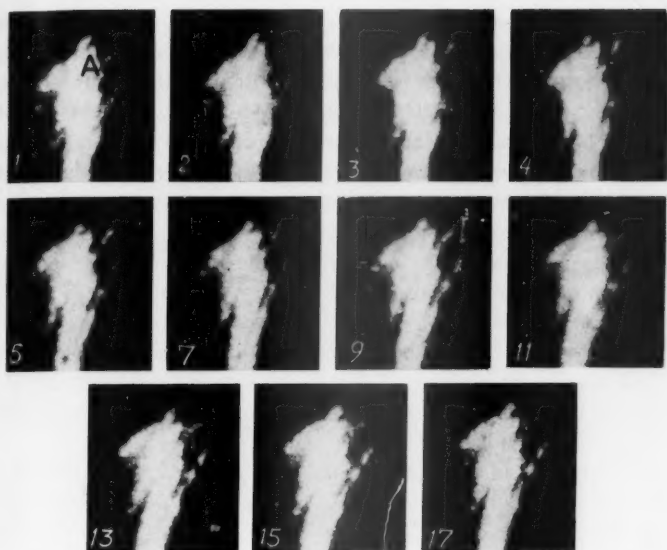


Figure 15. Photomicrographs from a film of the ciliary movement. 500 exposures per second. At A a point of light is discernible, which increases in extent until frame 4. It then diminishes and on frame 17 the point has roughly the same appearance as on frame 1.  
Magnification  $25.8\times$ .

The ratio between the rapid and the slower phase was thus 1:3. This value did not differ from that in unexposed rats.

\* \* \*

The purpose of the above-described experiments was to investigate if protracted exposure to sulphur dioxide would produce any alteration of the tracheal mucous flow and ciliary activity. The concentration of  $\text{SO}_2$  used was about 10 p.p.m., which is regarded as the maximum allowable concentration for an eight-hour working day.

The rate of mucous flow was considerably retarded in the groups of rats exposed to  $\text{SO}_2$ . In some rats no flow was demonstrable.

The ciliary activity was significantly reduced in the rats exposed to  $\text{SO}_2$  for 18 days while, remarkably, no such effect was discernible in the rats exposed for a longer period.

Considering the altered mucous transportation—sometimes very pronounced—demonstrated in all the exposed groups of rats, it would appear that changes in the physiologic environment primarily affected mucous flow. Ciliary activity was more difficult to influence. It should be pointed out that this same reaction relationship was demonstrated in the comparative *in vivo* and *in vitro* experiments and in the investigations concerning the significance of the duration of the experiments.

The findings in the present chapter emphasize further this relationship between the secretory and ciliary mechanisms.

The possibility of changes in the effective and recovery strokes was studied in three rats. A rhythmic pulsation was visible on all three motion picture records. From one film the ratio between the two phases of the ciliary cycle could be calculated. It did not differ from the ratio in healthy rats.

The changes in mucous flow and ciliary activity were registered immediately after cessation of  $\text{SO}_2$  exposure. It was therefore considered important to determine if the retardation showed any tendency to improvement in rats not examined until about a month after exposure. Each of the two groups used for this purpose comprised only three rats. In neither group, however, could any normalization of the mucous flow be detected. The diminished ciliary activity in the rats exposed to  $\text{SO}_2$  for 18 days also persisted.

By exposing a group of rats only to the air inflow of the exposure chamber it was ascertained that the exposure technique as such did not influence the functions investigated.

On first thoughts it may seem contradictory that changes should have taken place in the mucous flow while the frequency of the ciliary beat in some cases remained normal. Several factors, however, may have contributed to this circumstance. The most important of these probably was alteration of the composition and quality of the secretion.

The observations here made appear to be of great interest, as they indicate that slowing or cessation of mucous transportation may occur without alteration in the frequency of ciliary beat.

## VII. The significance for mucosal function of certain changes in the secretion

In the preceding chapter changes in the speed of mucous flow were shown to occur while the ciliary beat frequency remained normal. This reaction of the tracheal epithelium was presumed to be caused by altered composition or quantity of the secretion. The experiments to be reported in this chapter were made with the object of illustrating the influence of such alterations. The small amount of tracheal mucus in the rat renders chemical and physical investigation of the secretion extremely difficult. Hence the writer's experiments are not claimed to provide more than an orientation in the problems concerned. In addition to its quantity, the viscosity of the secretion, according to reports in the literature, is highly important for the velocity of flow.

### *The influence on mucosal function of the mucous blanket's thickness*

The depth of the mucous blanket may exert a direct influence on the power of the cilia and thereby also affect the mucous flow in the respiratory tract. With accentuated mechanical pressure on the cilia, as when the thickness of the mucous blanket is increased, the ciliary movement sooner or later must become less effective.

Systematic measurements of the depth of the tracheal secretion in normal or pathologic conditions do not appear to have been made. The writers who have discussed questions in this field have made only rough estimates. Hilding (1932) and Tremble (1947) both described the mucous blanket in the human nose as extremely thin, highly viscous and elastic. Proetz (1953) wrote that "the

mucous blanket has four essential attributes of clinical importance: it is exceedingly thin, slippery, adhesive and tenacious. It is so thin as to be invisible in the normal nose, although it may be picked up at any point, like a spider web, with a capillary pipette".

The thickness of the mucous blanket is mainly dependent on the activity of the secretory mechanism, i.e. the goblet cells and the mucous glands. Florey, Carleton and Wells (1932) in all probability demonstrated that stimulation of the recurrent laryngeal nerve and central parts of the vagus nerve brought about increased secretion from the glands of the trachea. This secretory increase could be inhibited by the administration of atropine. The goblet cells, on the other hand, could not be discharged either by nerve stimulation or by direct irritation of the mucosa.

Writers such as Lommel (1908), Lucas and Douglas (1934), Gordonoff and Mauderli (1936) and Ballenger (1949) studied the mucous blanket in pathologic conditions. All described how after, for instance, excessive heating, the cilia beat under a thick and viscous layer of mucus which moved slowly or not at all. From this Lucas concluded that the mucous blanket consists of two layers, a lower, serous in which the cilia function and an upper, more viscous layer. Sjöstrand (1941) pointed out that the secretory streaming is estimated to be insignificant as a rule at a distance from the cilia of four to five times their length.

Although the literature lacks systematic studies of the thickness of the mucous blanket in different circumstances, a number of experiments has been reported concerning the power of the cilia during increased load. Of such writers may be mentioned Bowditch (1876), Maxwell (1905) and Stewart (1948).

The last-named writer examined various ciliated membranes from the frog. The rate of transit of test objects was measured. These consisted of discs of aluminium foil. In one series of experiments the test objects were of uniform thickness but of different diameters. The weight per surface unit was thus constant while the area of contact varied. The speed of transportation was found to be constant and so independent of the diameter of the test objects. In another experimental series the area of contact was uniform but the load ranged from 0.008 to 140 mg per square mm. The speed of movement of these test objects was about the same up to a load

of 20 mg per square mm. Loads greater than this resulted in a rapid decline of velocity. Loads exceeding 140 mg per square mm often remained stationary. The relationship between load and speed of movement was approximately linear.

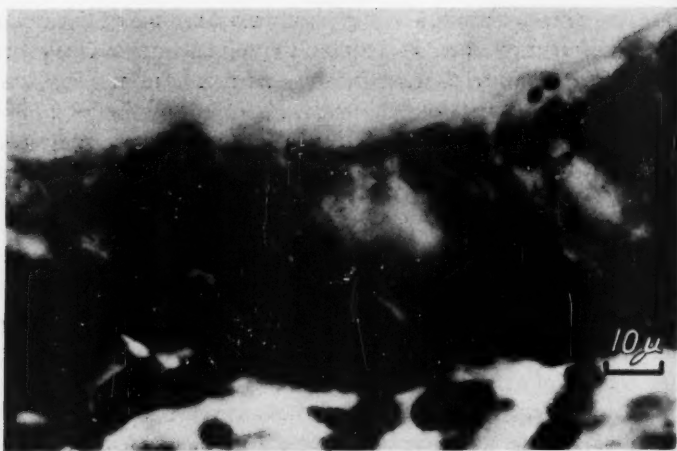
Among the studies of mucosal weight-carrying capacity should perhaps be counted those of Pease and Kitching (1939). They used *Mytilus edulis* and varied the hydrostatic pressure. A pressure of about 4 kg per square mm resulted in irreversible damage to the ciliary mechanism.

Certain determinations in the present study concerned the mucous blanket's thickness. In microscopic observation of the two groups of rats exposed to sulphur dioxide and described in Chapter VI, the amount of mucus in the trachea was clearly greater than in the normal rats. The mucous blanket in the latter rats would not have been discernible to microscopic observation if the secretion had not contained shed epithelial cells, etc. In the mucosal areas from which no direct light reflection proceeded, underlying blood vessels were distinctly seen in these animals. Such vessels, on the other hand, were not always distinguishable in the exposed rats.

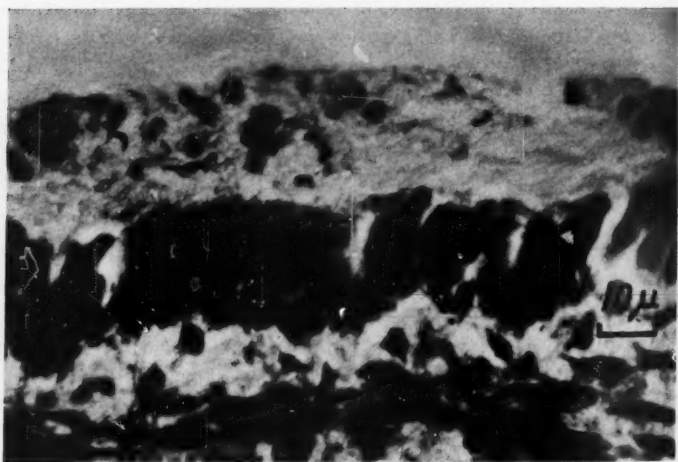
For measuring the depth of the mucous blanket the writer employed the freeze-fixation method evolved by Gersh (1932) and Hoerr (1936) and the apparatus described in detail by Sjöstrand (1944). Briefly, the principle of this method is as follows: Small pieces of tissue, removed from the animal less than one minute after death, are immersed in iso-pentane in a container with liquid air. The temperature in the iso-pentane bath is about  $-170^{\circ}\text{C}$ . The frozen tissue is then kept in a complete vacuum during one week, after which it is embedded in plastic and sectioned. A Spencer microtome as modified by Hillier and Gettner (1950) and Sjöstrand (1953 a) was used for sectioning in the present experiments. The preparations were stained with Heidenhain stain. The mucous blanket was easily visualized and its thickness readily measurable. It was also possible to entrap the cilia in different phases of their movement.

Three healthy rats were used in these experiments and one of the group exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$  (Chapter VI).

The mucous blanket in the healthy rats was about 5 microns in thickness, or about the length of a cilium (Figure 16). The secre-



*Figure 16.* Freeze-fixed mucosa from a healthy rat. The mucous blanket is distinguishable above the cilia. The midportion of the micrograph shows the cilia captured in various movement phases.  
Magnification  $800\times$ .



*Figure 17.* Freeze-fixed mucosa from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ . The mucous blanket is considerably thicker than in figure 16. The secretion contains numerous cellular elements.  
Magnification  $800\times$ .

tion appeared porous and loosely composed and contained only a few cellular elements. The micrograph from the exposed rat (Figure 17) illustrates the greatly increased thickness of the blanket as compared with the normal picture. After  $\text{SO}_2$  exposure the blanket was 20 to 25 microns thick, equal to the length of four to five cilia. The secretion also appeared more compact and contained numerous elements such as shed cells and white blood cells.

### *The influence on mucosal function of the secretion's viscosity*

The secretion in the human respiratory tract consists of mucin 2.5 to 3 per cent, salts 1 to 2 per cent and water 95 to 97 per cent (Tremble 1947, Proetz 1953).

Mucin is one of a group of substances called glucoproteins. Its main constituent is a complex carbohydrate radical combined with a protein. Glucoproteins occur widely in the plant and animal kingdoms and much labour has been expended on their chemical and physical classification (e.g. Hammarsten 1911, Levene 1925). The carbohydrate radical has been considered to be mucoitin-sulphuric acid. More recent studies (Werner 1953), however, have shown that in addition to mucoitin-sulphuric acid, complex polysaccharides, "fucomucans", are present in practically all mucous secretions. It has not been uniformly demonstrated to which of the constituent radicals the properties of viscosity are bound. Certain facts indicate that they are combined with the carbohydrate radical.

Small changes in the mucin percentage of the respiratory-tract secretion produce great variations in its viscosity (Linton 1933, Buhrmester 1936, etc.). The former writer stated: "an enormous increase in viscosity occurs in the concentration range between 3 and 5 per cent and a very appreciable increase between 2 and 3 per cent". The viscosity of respiratory-tract mucus from human subjects, has occasionally been investigated. In order to determine the viscosity of nasal mucus Buhrmester (1933, 1936) had to make a tenfold dilution in physiologic saline. This procedure cannot be regarded as satisfactory. Buhrmester herself stated that "the heterogeneous nature of the secretion and the rapidity with which it loses water are great handicaps in measuring viscosity directly".

Determinations of viscosity in general can be made by several

means. The extreme paucity of tracheal secretion in the individual rats, and perhaps above all the highly viscous nature of the mucus, rendered its collection exceedingly difficult in the present study.

Despite these difficulties the present writer, in consideration of the great importance of viscosity for the problems under investigation, decided to test some of the available methods.

Of the various techniques which have been described for viscosity determination, only micromethods could be contemplated for study of rat tracheal secretion. Of these latter may be mentioned the Hess viscosimeter, the method of Ligenza and Bernstein (1951) and the centrifuge method. In the present writer's trials of these methods, however, it was clear that they demanded dilution of the secretion. As such dilution was deemed unsatisfactory for the purposes in view, it was considered fruitless to continue the measurements.

## VIII. The reaction of the tracheal ciliary activity to single exposure to respiratory irritant gases and studies of the pH

The functional changes following protracted exposure to sulphur dioxide were reported in Chapter VI. While the rate of mucous flow was altered, no effect on the ciliary beat frequency was demonstrable in some cases. This finding prompted the writer to investigate if any change in the ciliary activity would occur after exposure to low concentrations of several respiratory irritant gases. The response of the cilia to ammonia, sulphur dioxide and formaldehyde will be discussed in the following pages. Experimental investigation was also made of possible changes in the tracheal secretion's hydrogen ion concentration during exposure. One of the reasons for the choice of these three gases was their potential effect on the pH. Alteration of the pH may conceivably influence the ciliary activity. Any shift in the pH due to ammonia would be towards alkalinity. Sulphur dioxide and formaldehyde could be expected to produce a shift to acidity, slight in the latter case as compared with sulphur dioxide.

The principles concerning the action of respiratory irritant gases were discussed by Haggard (1924). Although no personal experiments were reported, Haggard dealt generally with changes in the respiratory-tract epithelium following exposure to irritant gases. Thus he stated that the site of irritation is dependent on the solubility of the gas and that the first signs of such irritation are vasodilation and oedema. In serious cases the mucosa could be "lifted from the submucosa by fluid". The only special studies known to the present writer of the influence of irritant gases on the mucous flow and ciliary activity are those of Cralley (1942). The method employed, however, differed from that here used. Cralley's

experiments, for instance, were made *in vitro*. Thus it seemed important to perform corresponding investigations with the technique described in Chapter II.

Cralley studied *inter alia* the tracheal mucosa of rabbits after single exposure to various gases. He found that both formaldehyde and sulphur dioxide in concentrations of 20 to 30 p.p.m. brought about cessation of ciliary movement in approximately 60 seconds. The concentration of ammonia required to produce this effect was about 200 p.p.m. Cralley also observed the nasal mucous flow in man under normal conditions and during inhalation of various concentrations of sulphur dioxide. A drop of a red dye was placed in the inferior meatus while the subject breathed sulphur dioxide and the time taken for the dye to appear in the pharynx was recorded. Inhalation of 10 to 15 p.p.m. sulphur dioxide for 30 minutes resulted in a 10 to 15 per cent reduction of the speed of mucous flow, while 25 to 30 p.p.m. brought about a 45 to 50 per cent reduction. Cralley remarked: "exposure to small amounts of irritant gases for long periods of time may lead to a chronic irritation of the mucosa which would accordingly affect ciliary activity. Additional experimentation is required to evaluate this factor."

### *Method of exposure*

The desired concentration of the gas was prepared. It was then conditioned to 90 to 95 per cent relative humidity and about 34° C. For the humidifying process the gas was led through a washbottle with a fine-pored porcelain filter. The bottle was placed inside the moist chamber and the gas thus acquired the temperature of the chamber. The moistened and warmed air was then allowed to flow over the trachea of rats prepared and placed in the chamber in the usual way (p. 32). Care was taken in all the experiments to keep the same distance (2 cm) between the mouth-piece of the apparatus and the trachea, and to direct the stream of gas along the long axis of the trachea.

With the object of calculating a suitable velocity for the gas stream, the respiratory movements of three rats were registered kymographically. On the basis of these experiments the mean

velocity of air flow in the rat trachea was calculated to be about 7 centimetres per second, corresponding to 4 metres per minute.

By means of a flow meter the gas stream during actual exposure was adjusted to flow over the trachea at about 3 metres per minute. A lower flow velocity than that registered experimentally was chosen in order to avoid all influence of the streaming on the mucous blanket. The mouth-piece consisted of a glass tube 4 mm in diameter.

### *The reaction of the ciliary activity*

As stated in the introduction to this chapter, the writer wished to investigate the response of the cilia to single exposure to respiratory irritant gases. In this connection cinematographic recording with subsequent determination of the ciliary beat frequency was not undertaken. Nor did the problem concern the rate of ciliary beat, but could rather be expressed as follows: Would the cilia continue to beat after single exposure to these gases, or would ciliary movement cease? Continuous microscopic observation was made and the time registered from the beginning of exposure to the cessation of ciliary movement. Otherwise the technique and conditions were in conformity with those described on page 35.

With rare exceptions three healthy rats were used for testing each concentration of the various gases investigated. Three readings were made on each rat. Approximately ten minutes were allowed to elapse between each reading, so as to give the mucosa time to recover.

#### **"Air controls"**

For the first experiments with the above-described apparatus, only warmed and moistened air was used. The object was to determine if the air stream as such had any effect on the ciliary activity. After 10 minutes' treatment with air at 34° C and 90 to 95 per cent relative humidity, however, no effect on ciliary movement could be detected. Nor was there any sign of mucosal drying. The mucous flow proceeded with unaltered speed during the entire ten minutes in all three rats.

When it had thus been ascertained that the actual method of exposure, at any rate when continued for ten minutes, produced no demonstrable changes, the three above-mentioned gases were tested with the same technique.

### Ammonia

The ammonia preparation used was made by mixing ammonia from an ordinary container with air in the desired proportions.

The concentration of ammonia in the mixture was checked by the method described by Jacobs (1953). This method is applicable down to a content of 5 p.p.m.

The ammonia concentrations tested were 90, 45, 20, 10, 6.5, and 3 p.p.m. The last two experiments, however, were somewhat unsatisfactory, due to the uncertainty of the gas analysis method at low concentrations. For each of the concentrations 6.5 and 3 p.p.m. only two rats were used. All the other strengths were tested on three rats.

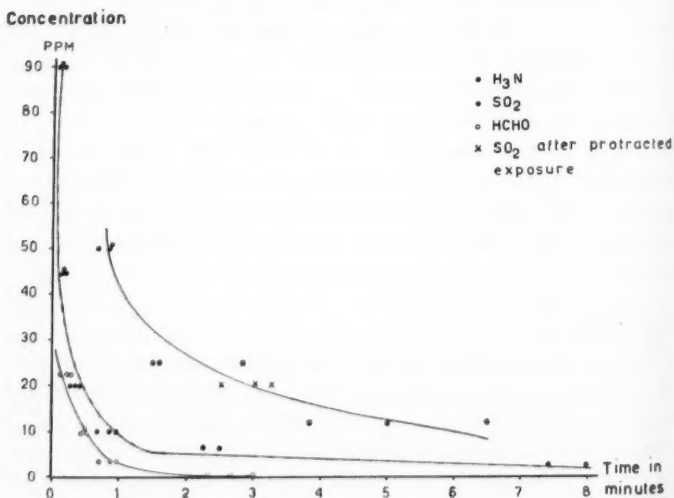


Figure 18. The time from the start of single exposure to cessation of ciliary movement. Each dot represents the mean of 3 determinations in one rat. As a rule, each concentration was tested on 3 rats.

The results are illustrated in Figure 18. It is seen that exposure to ammonia, even in low concentrations, very rapidly brought about cessation of ciliary activity. Recovery of mobility, however, was also very rapid—10 to 30 seconds after the exposure was terminated. It would also seem that the time before ciliary movement ceased was dependent on the ammonia concentration employed. Thus the cilia stopped beating after about 5 seconds' exposure to 90 p.p.m. ammonia, after 10 seconds with 45 p.p.m., after 20 seconds with 20 p.p.m. and after 150 seconds with 6 to 7 p.p.m. The corresponding time with 3 p.p.m. ammonia was 7 to 8 minutes. As already stated, the last two values are less reliable than the others, the method of gas analysis being less precise at such concentrations.

#### **Sulphur dioxide**

The mixture of sulphur dioxide and air was prepared in the same way as the ammonia gas, viz. by mixing sulphur dioxide from a container with air in suitable proportions.

The sulphur dioxide concentration was determined by a modification of the method reported by Jacobs (1953). Satisfactory analyses were thereby obtained at concentrations as low as 2 p.p.m. The action of sulphur dioxide on ciliary activity was tested with 50, 25 and 12 p.p.m.

The ciliary beating ceased in all the experiments, (Figure 18). Thus 50 p.p.m. sulphur dioxide resulted in stoppage of ciliary movement after about 50 seconds, while 25 p.p.m. took about 2 minutes and 12 p.p.m. about 4 to 6 minutes to produce the same effect. In these experiments, too, the cilia regained mobility a few seconds after the exposure ceased.

#### **Formaldehyde**

Formaldehyde was prepared as follows: An electric heating plate was placed in a fairly wide glass tube. This tube was introduced into a plastic bag with a capacity of about 160 litres (Figure 19). A weighed quantity of paraformaldehyde was placed on the plate. By heating the plate the paraformaldehyde was vaporized into the bag. Simultaneously the desired volume of air was pumped into the bag. Such plastic bags are unsuitable for long-term storage of formaldehyde, as the gas diffuses out and the concentration is

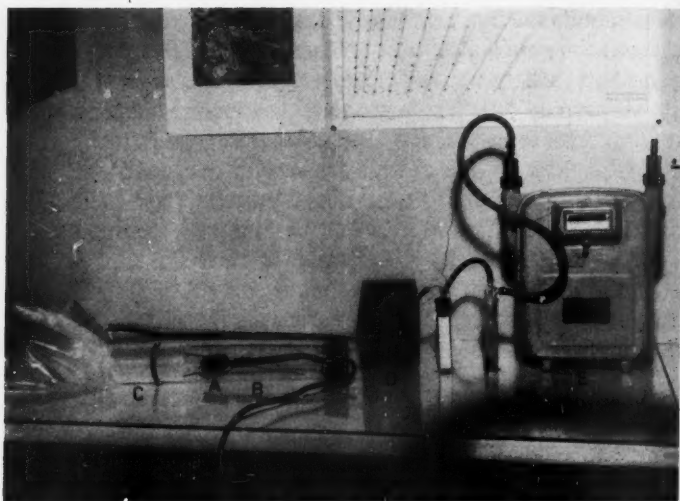


Figure 19. A. Electric heating plate. B. Glass tube. C. Plastic bag. D. Pump. E. Flow meter.

thereby diminished. As the experiments were carried out within 10 to 15 minutes, however, the gas loss was not of an order requiring consideration in the present connection. In a control test the gas concentration in the plastic bag was found to diminish from 200 to 180 p.p.m. in 3 hours.

For determination of the formaldehyde concentration a method described by Barnes and Speicher (1942) was employed. This method is applicable down to approximately 0.5 p.p.m.

The formaldehyde concentrations tested were 22, 10, 3 and 0.5 p.p.m. The approximate interval before ciliary movement ceased was 10 seconds with 22 p.p.m. formaldehyde, 30 seconds with 10 p.p.m. and 50 seconds with 3 p.p.m. With 0.5 p.p.m. the ciliary movement, and therewith also the mucous transportation, stopped in about two and a half minutes (Fig. 18). The beating recommenced, however, 10 to 30 seconds after the exposure was terminated.

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The above findings prompted the question whether or not ciliary movement ceases in rats which breathe  $\text{SO}_2$  normally, i.e. without tracheotomy. From experience with the experiments hitherto described in this chapter, it was evident that the question could not be answered with the writer's preparation technique. Attempts were nevertheless made to prepare anaesthetized rats during continuous administration of  $\text{SO}_2$  and with the least possible delay place them in the moist chamber. The time from opening the trachea to placing the rat in the chamber and focusing a suitable mucosal "high-light" could not be reduced to less than approximately one minute. No effect on the ciliary beat frequency could be observed in rats so prepared. In order to demonstrate a possible effect of normally-breathed  $\text{SO}_2$  the preparation time would have to be brought down to less than 10 to 30 seconds, and this does not appear to be feasible.

Yet another question of interest is whether or not rats exposed for long periods to, for instance,  $\text{SO}_2$  become in some way habituated to the gas, so that the ciliary response to its administration is less rapid.

Three rats which had been exposed for 48 days to 10.4 p.p.m.  $\text{SO}_2$  (p. 84) were therefore subjected by the above method to single exposure to 20 p.p.m.  $\text{SO}_2$ . No difference was then observed in the response of the ciliary movement as compared with the findings from single  $\text{SO}_2$  exposure in previously unexposed rats. Thus the ciliary movement ceased after about 3 minutes (Figure 18) and very rapidly recommenced when the exposure terminated. The corresponding time in the rats not previously exposed was 2 minutes with a concentration of 25 p.p.m.  $\text{SO}_2$ .

### *Studies of the secretion's pH*

The primary factor in the rapid cessation of ciliary activity during single exposure to irritant gases must have been dissolution of the gases in the secretion, with subsequent effects on the mucosa. In regard to the solubility in water of the three gases used, ammonia is about 40 times more soluble than sulphur dioxide but about 50 times less so than formaldehyde at the same pressure and temperature. The solubility ratio of sulphur dioxide, ammonia and formalde-

hyde is thus 1:40:2,000. The suppression of ciliary activity could conceivably have been brought about by a shift in the secretion's hydrogen ion concentration, by influence of the specific ions which arise when the gas dissolves in the secretion, or by a combination of these factors. Experiments were therefore performed with the object of shedding some light on possible changes in the pH during the above-described exposures. The action on the mucosal pH of protracted exposure to  $\text{SO}_2$  was also investigated.

Before describing the experiments, however, the relevant literature will be briefly reviewed. Some studies of the pH of the ciliary environment will first be discussed and thereafter methods of determining the secretion's pH.

Gray (1928) showed that cilia from *Mytilus edulis* ceased to beat when the pH of their environment was reduced to 5.0. Between pH 6.4 and 7.6 the rate of beat rose sharply and remained constant up to pH 9.2.

Tomita (1934) studied the gill cilia of oysters and showed a steep fall in activity at pH 5.5. Negus (1934) found that the cilia in extirpated rabbit membranes could not continue to beat when the pH was lower than 6.4.

In connection with the effect of pH on the ciliary activity, the influence of several acids has been reported in the literature. Those acids which rapidly penetrate into the cells, e.g. HCl, appear to have the speediest effect on ciliary activity (Haywood 1925, Yonge 1925, and Linton 1933).

Measurement of the mucosal secretion's pH has been made directly on the mucosa *in situ* or *in vitro* on collected secretion. Although the latter method was not applicable in the present investigation, some findings with it will be reviewed.

*In vivo* measurements were made by Mittermaier (1930), Fabricant (1941), Nungester and Atkinson (1944), and Dietz (1944). All these writers measured the pH in the nasal cavities of man or laboratory animals with the aid of an electrode placed directly on the mucosa. Nungester and Atkinson recorded a mean pH of 7.0 and Dietz 7.2. Fabricant's normal reading was lower—6.2.

Parkinson (1945) strongly criticized these investigations. He maintained that as the intranasal position of the electrode was not satisfactorily accounted for, it was not possible to determine if

the recorded pH derived from the tissue or from the secretion. The two values are not necessarily identical.

Colorimetric tests of the intranasal pH were made by Tweedie (1934). Cotton-wool swabs moistened with bromthymol blue or cresol purple were applied to the nasal mucosa of human subjects with the aid of a sound. By comparing the reactions of these indicators with a standard colour index, Tweedie found the normal pH of the nose to be 6.8 to 7.4.

From determinations *in vitro* on human nasal secretion Hilding (1934) recorded a normal pH of 7.2 to 8.5 and Buhrmeister (1933, 1938) 7.4 to 7.9.

#### **The mucosal pH during single exposure to sulphur dioxide**

The rat trachea contains scarcely 1 cu.mm of secretion. As earlier stated, this fact and the viscosity of the mucus made its collection extremely difficult. Further, the exceeding thinness of the normal mucous blanket made it impossible to place an electrode with satisfactory precision on the mucus, while avoiding contact with the actual membrane.

With the studies reported below the writer tried to attain conditions resembling those in the trachea as regards the mucous blanket and its thickness.

A round flask with a capacity of 1,000 c.c. was provided with inflow and outflow pipes. Into the flask was pipetted in one series of experiments 5 c.c. of a physiologic sodium chloride solution. By rotation of the flask the fluid was fairly evenly distributed around its walls. The depth of the fluid surface so formed was approximately estimated to 0.1 mm. Sulphur dioxide was then supplied through the inflow pipe in various concentrations and for various periods. The changes in the pH of the solution were recorded. The approximate SO<sub>2</sub> concentrations used were 15, 30 and 50 p.p.m. The duration of exposure to each concentration was 30 seconds, and 2, 5 and 10 minutes. Three tests were performed with each concentration and exposure period.

The pH measurements were made with a Radiometer pH-meter 22. Double readings were made for each test. No difference was recorded between the readings.

The initial pH of the saline solution was 6.6. As expected, the  $\text{SO}_2$  was very rapidly dissolved and the pH of the solution fell. With 50 p.p.m.  $\text{SO}_2$  the pH of the solution fell to about 3.0, irrespective of whether the exposure was for 2 or 5 minutes. Continued supply of  $\text{SO}_2$  resulted in very little further pH fall. Even when a relatively low concentration of  $\text{SO}_2$  was supplied, the pH of the saline solution fell after about 2 minutes to a value which was not notably altered by further exposure.

With the object of approaching more closely the conditions in the respiratory tract the writer, in another series of experiments, added mucin in a strength of 2.5 per cent to the physiologic sodium chloride solution. According to the literature (e.g. Proetz 1953), the percentage of mucin in the respiratory-tract secretion is 2.5. The only mucin available was gastric mucin, supplied by L. Light & Co. Ltd., London. In all probability this mucin differed in composition from mucin in the rat respiratory tract. The intention, however, was to investigate if mucin would counteract the pH-reducing action of sulphur dioxide. The buffering capacity of mucin has been demonstrated by several authors (e.g. Blix 1950).

The initial pH of the mucin solution was 4.6. When this solution was exposed to the same concentrations of  $\text{SO}_2$  for the same periods

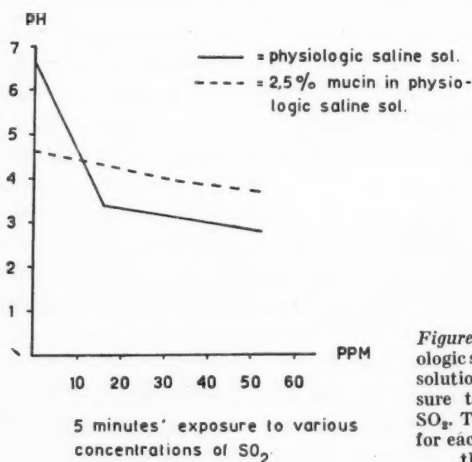


Figure 20. The pH of physiologic saline solution and mucin solution after 5 minutes' exposure to 15, 30 and 50 p.p.m.  $\text{SO}_2$ . Three readings were made for each gas concentration and the mean calculated.

as in the preceding experimental series, its pH showed only a comparatively small and slow reduction (Figure 20). After 10 minutes not even the strongest  $\text{SO}_2$  concentration had brought about a pH fall of the order registered when sodium chloride solution alone was exposed for only 2 minutes to the same gas concentration. Tests were also made with 2.5 per cent mucin in Tyrode's solution and with Tyrode's solution alone. The initial pH of this mucin solution was 7.4 and that of the Tyrode's solution 8.4. After 5 minutes' exposure to 52 p.p.m.  $\text{SO}_2$  neither of these values had altered by more than one decimal point. The respective pH readings were then 7.3 and 8.3.

An attempt at maximum saturation was made by allowing 10.2 p.p.m.  $\text{SO}_2$  to bubble through mucin solution and physiologic sodium chloride solution for 30 minutes. The pH of the former fell from 4.6 to 4.4 and that of the saline solution from 6.6 to 3.8.

Further attempts to explore the effect of various ions and pH values were not considered necessary in the present connection, the primary interest being the action of the gas used in the experiments with protracted exposure.

#### **The mucosal pH during protracted exposure to sulphur dioxide**

Because of the difficulties in collecting tracheal secretion and in satisfactorily placing an electrode on the membrane, the writer elected to make colorimetric determination of the pH of the tracheal secretion in normal rats and after exposure to  $\text{SO}_2$ .

A reagent solution was dropped directly onto the opened trachea of rats prepared in the usual way. The rats were then placed in the moist chamber. The reagent used was bromthymol blue which has an indicator range from pH 6.0 to 7.6. Its own pH is 7.4. This reagent was chosen as having a well-defined interval which is close to the region of the putative normal pH values of tracheal secretion in the literature. Bromthymol blue is normally dark blue. It changes via bluish-green and greenish-yellow to clear yellow when the pH is less than 6.0. Above pH 7.6 its colour is still blue.

Bromthymol blue was dropped directly onto the mucosa of five healthy rats. All showed uniformity of reaction. The blue stain

of the reagent was fairly rapidly superseded by a blue-green to green-yellow stain, but not by clear yellow. Exact observation of the shades of stain was not feasible because of the underlying membrane's colour. That a change to clear yellow did not take place, however, was obvious from the fact that, when a drop of 0.1 N HCl was added to the indicator solution in the trachea, a distinct clear yellow stain appeared. It was also obvious that the dark-blue colour of the indicator, which at first was clearly distinguishable against the mucosa, did not persist.

It would therefore seem probable that the normal hydrogen ion concentration of rat tracheal secretion lies within the pH range of bromthymol blue, viz. between 6.0 and 7.6.

Three of the *exposed rats*—10.4 p.p.m. SO<sub>2</sub> for 48 days—(Table 20, p. 84) were studied in regard to tracheal pH. Immediately following SO<sub>2</sub> exposure these rats were prepared and bromthymol blue dropped onto the tracheal mucosa. In no case were the reactions noted to differ from those in the healthy rats. The reagent thus took on a blue-green to green-yellow colour.

Within the limitations of the method employed, therefore, the pH of the tracheal secretion in the rats exposed to SO<sub>2</sub> showed no deviation from the values in healthy rats.

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The experiments presented in this chapter were designed to investigate how the cilia react when briefly exposed to some respiratory irritant gases and to shed light on possible changes in the hydrogen ion concentration of the mucous secretion.

The method employed for exposure cannot be directly compared with normal breathing of the gases concerned. Under normal circumstances the first ciliary reaction takes place in the nose. The extent to which the tracheal and bronchial epithelium is exposed to the inhaled gas then depends on a number of factors, such as the concentration of the gas and the period of exposure. It may be considered probable, however, that the ciliary reaction is basically similar whether the gas encounters only the nasal epithelium or

penetrates further into the respiratory tract. The experiments with protracted exposure to sulphur dioxide showed that this gas at any rate extends its detrimental effects at least as far as the trachea.

A flow of moistened and warmed air for ten minutes did not give rise to any discernible effect on ciliary activity. Consequently, the changes following exposure under the same conditions to ammonia, sulphur dioxide and formaldehyde were considered to derive from these gases.

The discrepancies in the period of latency for the various gases were probably ascribable to differences in solubility. The solubility ratio of sulphur dioxide, ammonia and formaldehyde is 1:40:2,000. It was interesting in this connection to note that sulphur dioxide evoked the slowest ciliary response. By comparison the reactions to ammonia and formaldehyde appeared very rapidly.

In regard to the hydrogen ion concentration the following may be stated. The gas, formaldehyde, which could be expected to produce the smallest shift in the secretion's pH, elicited the most rapid ciliary reaction. This may be associated with the greater solubility of formaldehyde. The mode of ciliary reaction, however, may also be interpreted as indicating that the pH is not the only responsible factor, and that other ions formed when the gases go into solution also play a part. This reasoning is further supported by the fact that mucin has a powerful buffering action, which was experimentally demonstrated. After 10 minutes' exposure to about 50 p.p.m.  $\text{SO}_2$ , the pH of a mucin solution was not reduced as much as that of physiologic sodium chloride solution following 2 minutes' corresponding exposure. After only 30 seconds' exposure to 30 and 50 p.p.m.  $\text{SO}_2$  the pH fall in the saline solution was greater than in the mucin solution when exposed for 10 minutes to the same gas concentrations.

From a purely practical aspect the reported findings indicate that brief exposure to at any rate the gases here employed, rapidly brings about suppression of ciliary movement in the affected areas. This also entails stoppage of the mucous flow during the exposure period.

Habituation to the effects of  $\text{SO}_2$  was not demonstrated in experiments in which three rats previously exposed for 48 days to 10.4 p.p.m.  $\text{SO}_2$  were exposed once to 20 p.p.m. The response of the ciliary movement did not differ from that in rats without previous exposure.



## MORPHOLOGIC STUDIES

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The effect of irritant gases on the respiratory-tract epithelium cannot be expected to be confined to purely functional changes. It must also be reflected in reactions which may be studied morphologically. Hence it was considered important to investigate the morphologic basis of the functional findings described in the foregoing.

For this purpose the emphasis was placed on ultrastructural studies, but light microscopy was also employed.

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## IX. The morphology of the tracheal mucosa in healthy rats and after protracted exposure to sulphur dioxide

In the section on functional investigations a series of experiments was presented in which the results indicated that exposure to sulphur dioxide was not necessarily followed by damage to the mechanism of ciliary movement. The speed of mucous flow, on the other hand, was reduced.

Because of these findings the normal morphologic picture of the tracheal epithelium was studied and a search was made for changes following exposure to sulphur dioxide. Particular interest was attached to the ultrastructure of the ciliated cells. This seemed especially warranted since, while gross changes of the epithelium and submucosa may be detected with the light microscope, a study of the fine structure of the ciliated cells, and of possible minute alterations therein, requires electron microscopy.

### *Methods*

The tissues examined with the light microscope and those used in electron microscopy were *extirpated*, *fixed* and *embedded* in the same way.

The trachea was *extirpated* under anaesthesia. The first sections were placed in osmium tetroxide solution only 10 to 15 seconds after removal from the animal. The *fixation* (Palade 1952, modified according to Sjöstrand 1953 a) and *embedding* (Newman, Borysko & Swerdlow 1949) of the tissues was continued with the technique now generally employed for electron microscopy at the Department of Anatomy, Karolinska Institutet.

For *sectioning* the tissues examined by light microscopy a Spencer microtome as modified by Hillier and Gettner (1950) and Sjöstrand (1953 a) was used. The blocks for electron microscopy were sectioned with the ultramicrotome designed by Sjöstrand (1953 b). The thickness of the sections cut with the Spencer microtome was as a rule 0.3 microns and of those cut with the ultramicrotome 0.01 to 0.02 microns (100 to 200 Å).

The light microscope was a Leitz "Ortolux". Phase contrast was used throughout. The objective lens was an oil immersion HI 90  $\times$ ; aperture 1.15, and the ocular a Periplan Ok P 8  $\times$ .

The electron microscope was an RCA EMU 2c model. The technique in these studies was in conformity with that employed by Sjöstrand (1953 a).

The examined tissues were taken from the following animals:

The "*controls*", comprising four healthy rats. In all of them the tracheal epithelium was studied with the light microscope and in three also with the electron microscope.

The "*air controls*" were three of the six rats exposed only to the air inflow of the exposure chamber for 62 days (p. 79, Table 14).

The "*exposed rats*" consisted of all nine exposed to 11.4 p.p.m. SO<sub>2</sub> for 18 days (group 1, p. 81, Table 16 and 17) and all nine exposed to 11.5 p.p.m. for 67 days (group 2, p. 82, Table 18 and 19).

*Light microscopy* of the tracheal epithelium was carried out in the three air controls and in all the exposed rats. In each of groups 1 and 2 six rats were examined immediately after SO<sub>2</sub> exposure and the remaining three not until 33 days later. *Electron microscopy* was performed in the three air controls, directly following SO<sub>2</sub> exposure in three rats from group 1 and six from group 2, and after 33 days in the other three rats from group 2.

### *The morphology of the tracheal mucosa in healthy rats*

#### **Light microscopy**

Figures 21 and 22 show that ciliary cells are the most common type. In these cells the basal corpuscles lie just below the cell boundary facing towards the tracheal lumen (Figure 21). A collection of mitochondria is seen near to and immediately under the basal corpuscles. The length of the ciliary cells was calculated from ten

cells in each of three rats; the mean length was 17.3 microns. The mean length of the cilia in ten estimations from each of the same three animals was about 4.3 microns.

Among the ciliary cells are interspersed smaller numbers of goblet cells. It is difficult to calculate the ratio of goblet cells to ciliary cells. Determinations made in the animals used for estimations of ciliary length indicated that for each goblet cell there are three to five ciliary cells.

The third type of cell in the tracheal epithelium is the basal cell. These are seen on the basement membrane, under and interspersed between the other cells (Figure 22).

Close under the basement membrane lies the lamina propria, rich in elastic tissue, blood vessels and glands.

#### Electron microscopy

The normal ultrastructure of the ciliated cells, and especially of the cilia, is of most interest in the present connection. For the sake of clarity, however, a brief description will be given of the structure of the other epithelial cellular components. For a more detailed presentation the reader is referred to Rhodin and Dalhamn (1956).

The cytoplasm of the *ciliary cells* appears on electron microscopy to be loose and finely granulated (Figure 23). Apical to the nucleus is the Golgi apparatus (Dalton and Felix 1954, Sjöstrand and Hanzon 1954 a, Rhodin 1954). The mitochondria, which are surrounded by a double membrane, are present in all regions of the ciliary cells, but are particularly plentiful in their apical portion, under the basal corpuscle. The structure of these mitochondria differs from previously observed normal types (Sjöstrand 1953 c, Sjöstrand and Rhodin 1953, Sjöstrand and Hanzon 1954 b, Rhodin 1954) in that the internal membranes, in addition to being double-lined, are mutually connected.

Rhodin and Dalhamn (1956), in 115 square microns of rat tracheal mucosa, comprising 15 cells, found the number of cilia per square micron to be 8.4. The mean length of the cilia was about 5 microns and their mean diameter 0.24 microns.

A cilium consists of a free portion outside the cell and a basal corpuscle just below the cell boundary. In cross sections (Figure 24) the free portion of a cilium is seen to comprise two central fila-

ments and, in a ring around them, nine peripheral filaments. Lateral and fairly close to these nine is the outer sheath of the cilium, which measures about 70 Å in thickness.

The two central filaments are clearly separate. They are formed of an outer opaque zone about 60 Å thick, surrounding a less opaque central portion. The whole filament is about 300 Å in diameter. The structure of the peripheral filaments is similar. Each has two component subfilaments in close contact. Each subfilament consists of an outer opaque zone and a less opaque centre. The thickness of the outer zone is about 60 Å and of the whole subfilament about 300 Å.

Longitudinal sections of the cilium (Figure 25) show no change in the interrelationship of the above-described filaments until the extreme tip of the cilium (Figure 26). There the peripheral filaments appear to fuse. This observation tallies with the report of Engström and Wersäll (1952). Cross sections at successively deeper levels of the cilium, towards the cell boundary and into the basal corpuscle, show the structural changes illustrated in Figure 27.

The first of these changes is that all the peripheral filaments converge to form a wreath-like structure. This seems to be brought about by lessening of the distance between the filaments and division of these into more subfilaments. At this level the central filaments can no longer be distinguished. The next change is seen immediately below the cell surface, where the surface membrane of the cilium is continuous with the cell membrane. Here the above-mentioned wreath-like structure encloses a weakly osmiophilic inner substance.

Still further down in the cell the space occupied by this substance becomes filled by further fusion and division of the peripheral filaments. In this region cross section of the basal corpuscle shows a raspberry-like pattern. From the lower pole of the basal corpuscle emanates in many sections a number of extremely thin rootlets. These can be traced into the cell only for about 0.5 micron. In longitudinal sections of the basal corpuscle the interior zone consists mainly of a weakly osmiophilic substance within which may be seen a kidney- or bean-shaped opaque body (Figure 28). This body is shown in cross section in Figure 27.

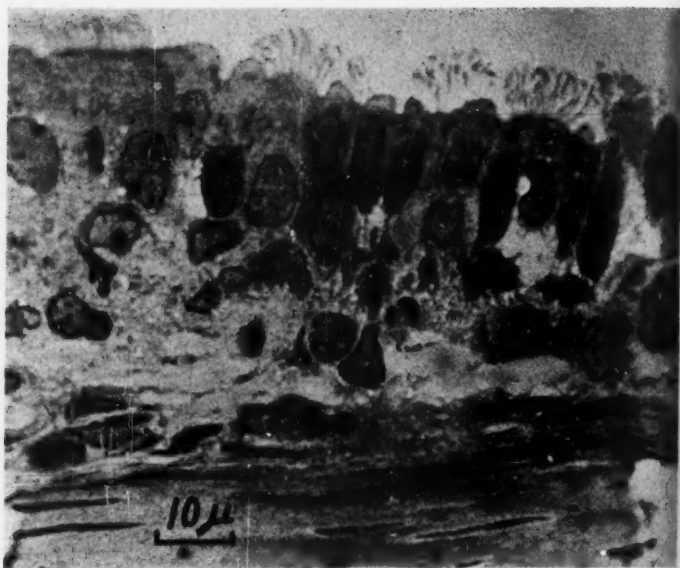
In the other main type of tracheal epithelial cell, the goblet cell,

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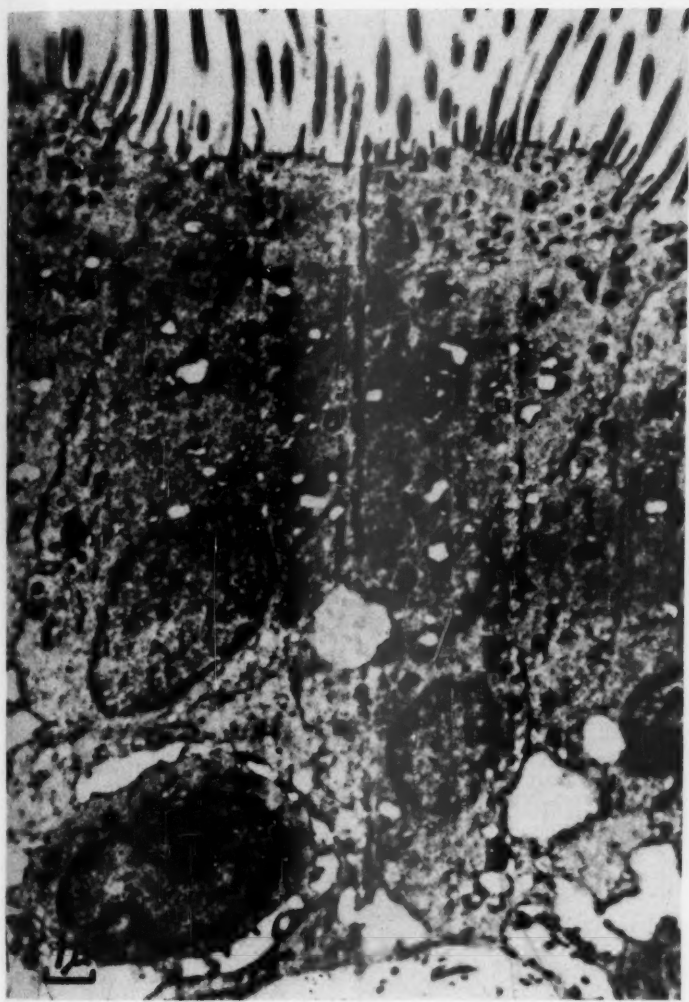


Figure 21. Section of normal tracheal epithelium. Interspersed among the ciliary cells are goblet cells in various phases of secretion. Beneath the ciliary and goblet cells are basal cells. The basal corpuscles lie close under the cell surface which faces towards the tracheal lumen.

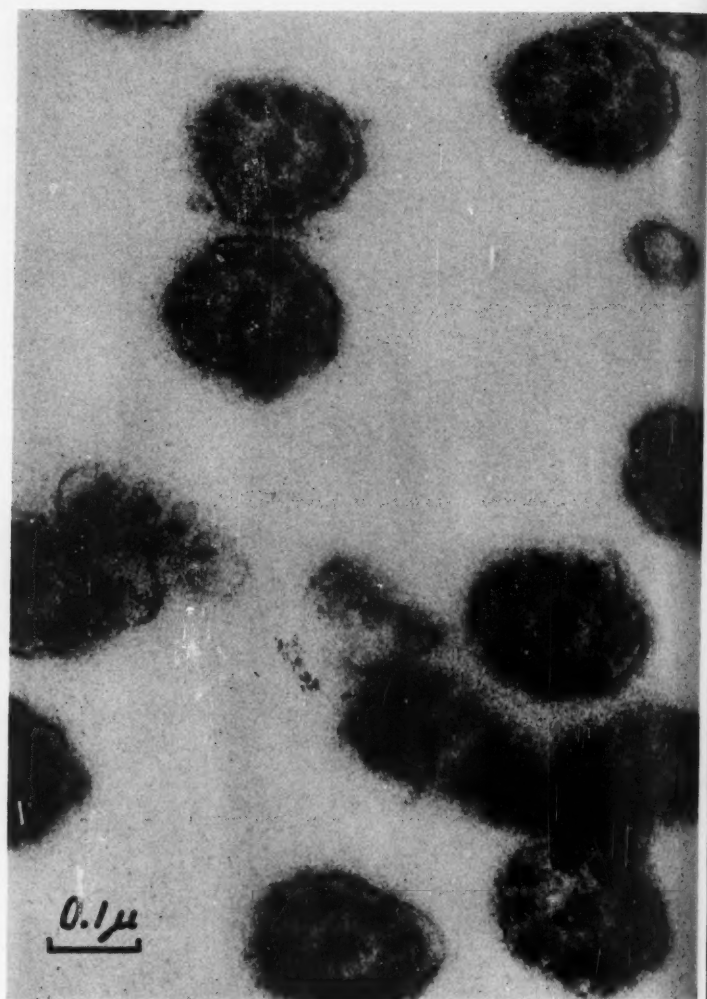
Phase contrast. Magnification 2,800  $\times$ .



*Figure 22.* Section of normal tracheal epithelium. Close beneath the basement membrane is the lamina propria.  
Phase contrast. Magnification 1,110  $\times$ .



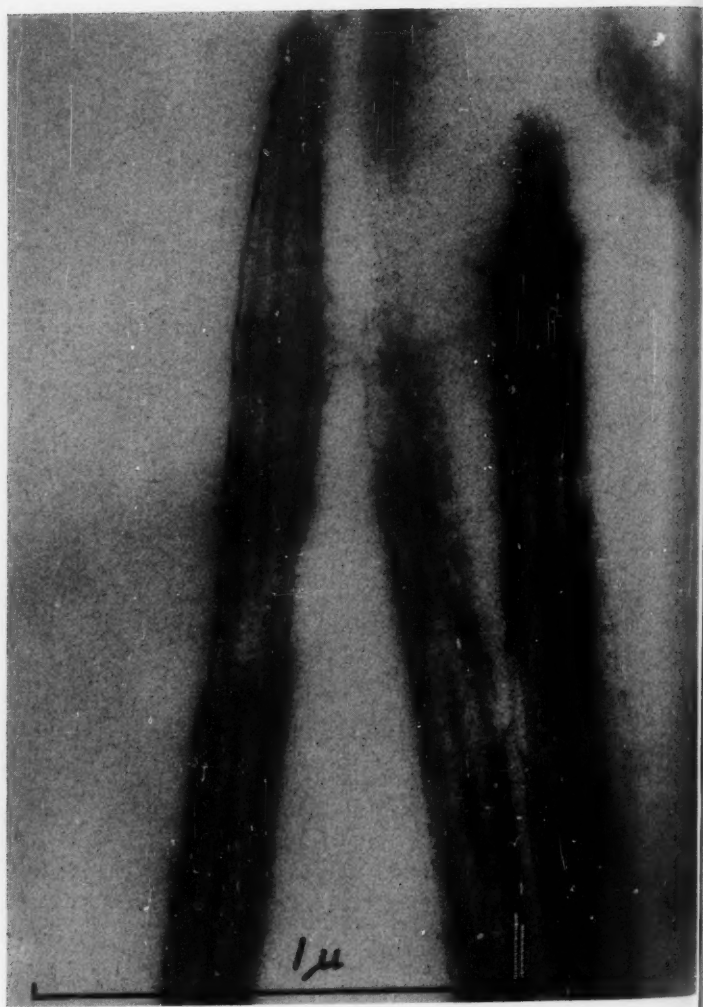
*Figure 23.* Ciliary cells from healthy rats. The cytoplasm is loose and finely granulated. The mitochondria are agglomerated just below the basal corpuscles.  
Below the ciliary cells is a basal cell.  
Electron micrograph. Magnification 7,200  $\times$ .



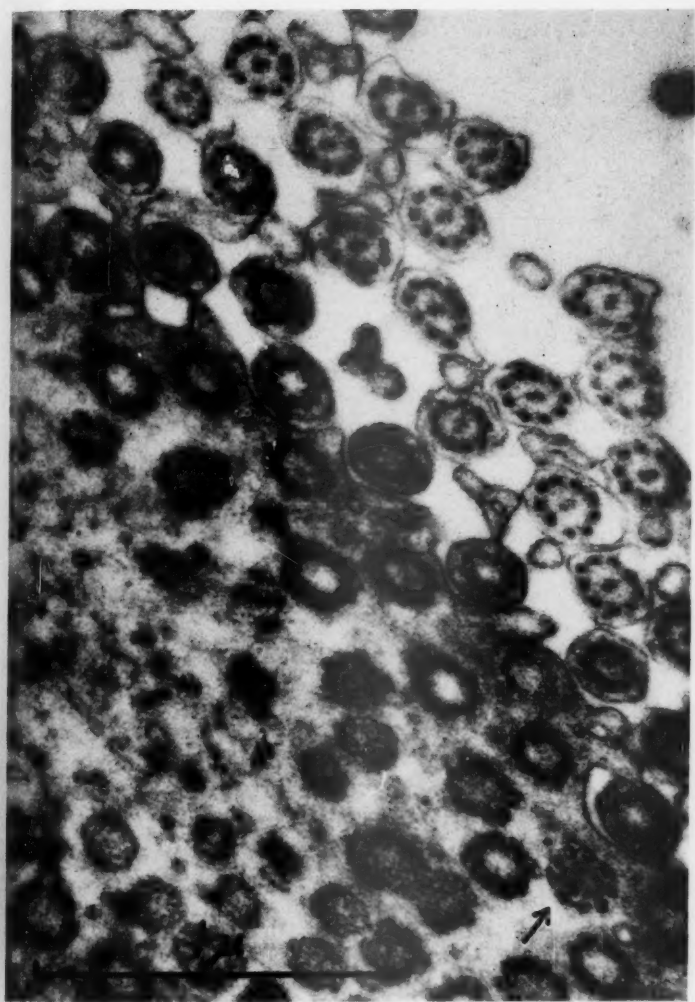
*Figure 24.* Transverse section of cilia. Two central filaments are surrounded by nine peripheral filaments. The cilium is enclosed in a thin sheath. From RHODIN and DALHAMN 1956.  
Electron micrograph. Magnification 133,800  $\times$ .



Figure 25. Longitudinal section of cilia, showing the central and the peripheral filaments. The course of the latter can be followed down into the basal corpuscle.  
Electron micrograph. Magnification 32,900  $\times$ .

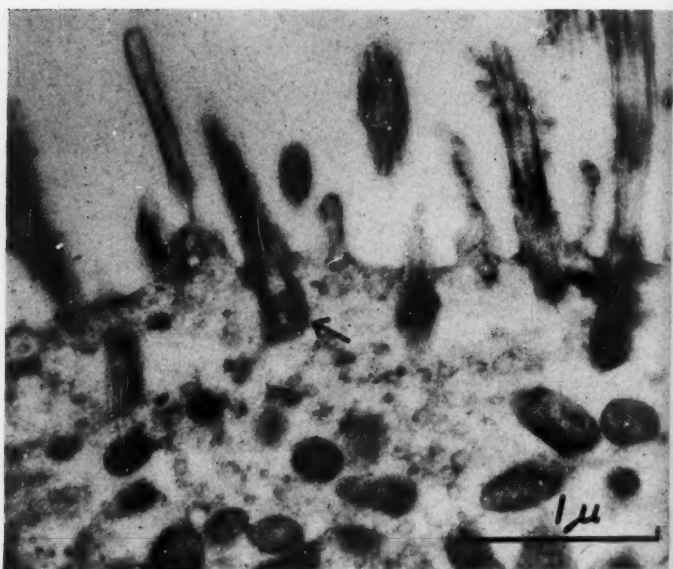


*Figure 26.* Longitudinal section of ciliary tips. The peripheral filaments appear to fuse. From RHODIN and DALHAMN 1956.  
Electron micrograph. Magnification 78,400  $\times$ .



*Figure 27. Transverse sections of cilia, taken at various levels. The peripheral filaments converge as the cell is approached and finally form a raspberry-like pattern. The arrow indicates the body illustrated in Figure 28.*

Electron micrograph. Magnification 57,100  $\times$ .



*Figure 28.* Longitudinal section of cilia and basal corpuscles, showing a kidney- or bean-shaped body (arrow). Under the basal corpuscles are agglomerations of mitochondria.  
Electron micrograph. Magnification  $29,600\times$ .

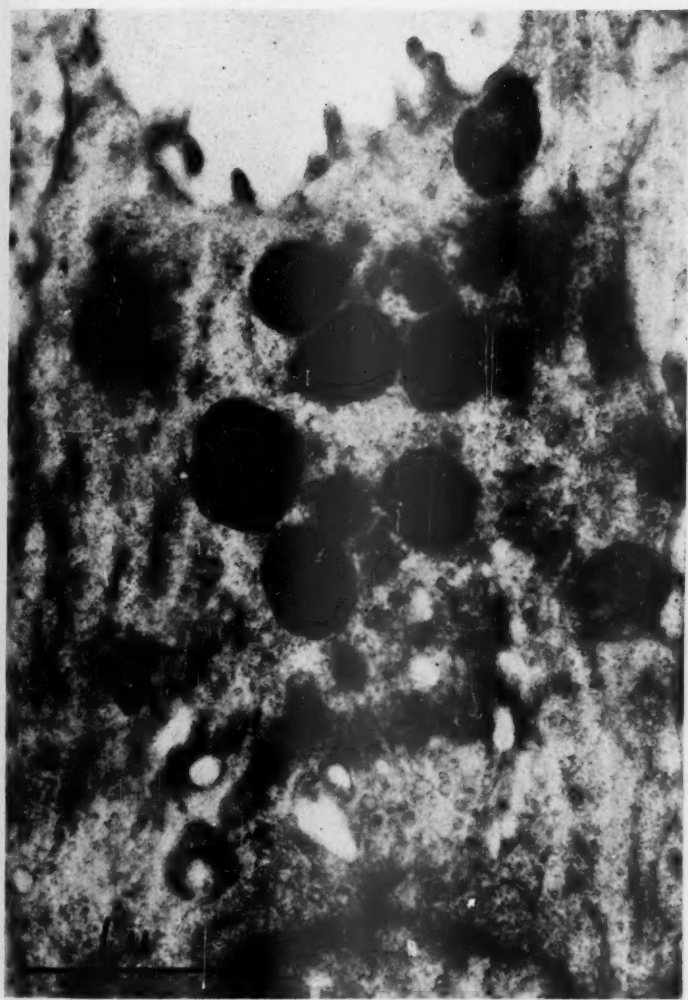


*Figure 29.* Transverse section of the tracheal epithelium at the level of the nuclei. The cytoplasm of the goblet cells is considerably more opaque than that of the ciliary cells.

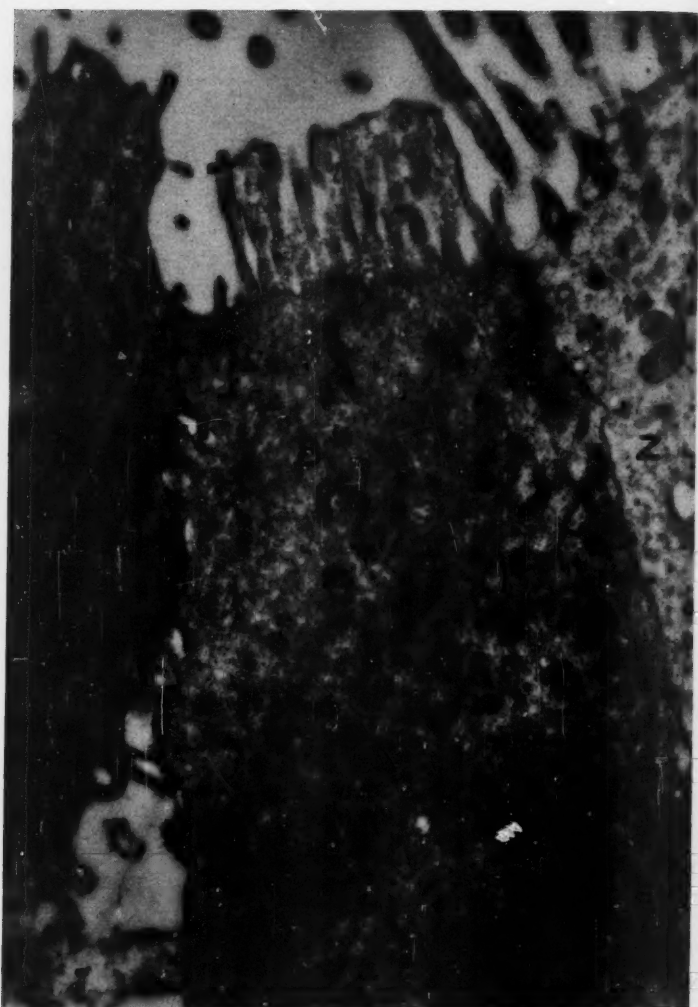
Electron micrograph. Magnification 10,600  $\times$ .



*Figure 30.* Longitudinal section of a goblet cell, showing a number of mucous granules. In the cytoplasm is a system of "α-cytomembranes".  
Electron micrograph. Magnification 13,400  $\times$ .



*Figure 31.* This goblet cell contains a number of highly opaque granules. Apical to the nucleus is the Golgi apparatus. From RHODIN and DALHAMN 1956. Electron micrograph. Magnification 31,100  $\times$ .



*Figure 32.* Longitudinal section of a "brush cell" B. The cell surface shows a number of protoplasmic projections, 1—1.5 microns in length. G a goblet cell, Z a ciliary cell and I an intercellular spatium. From RHODIN and DALHAMN 1956.

Electron micrograph. Magnification 15,750  $\times$ .

the cytoplasm is considerably more opaque than that of the ciliary cells (Figure 29). The mitochondria of the goblet cells do not differ in structure from earlier-described types. The cytoplasm shows a system of membranes (Figure 30). Some goblet cells are large and spherical and are filled with mucous granules which they discharge into the lumen of the trachea. Other goblet cells are more slender and do not appear to contain mucous granules. Instead there is a number of large, highly opaque granules (Figure 31).

To a third type of cell contained in the tracheal epithelium Rhodin and Dalhamn (1956) applied the term "brush cell" (Figure 32). This cell has an opaque cytoplasm, small mitochondria and, in its basal region, strongly osmiophilic granules with a markedly symmetrical structure. The brush cells, as the name implies, have on their surface a number of protoplasmic projections 2 to 3 microns in length. The cells lie close to one or more goblet cells.

The *basal cells* will not be further described here as they are of very limited interest for present purposes.

### *The morphology of the tracheal mucosa after protracted exposure to sulphur dioxide*

#### **Light microscopy**

In the *exposed* rats the light micrographs were of uniform appearance. The surface of the epithelium was no longer even; instead its cells were of unequal height. In places the epithelium displayed deep crypt formation. Such crypts were seen in the rats exposed to sulphur dioxide for 18 days (group 1, Figure 33) as well as in those exposed for 67 days (group 2, Figure 34).

The ciliary cells were considerably longer and more slender than normal and appeared compressed. The mean length of ten cells from each of three exposed rats was 25 microns. The corresponding figure for healthy rats was 17 microns. The cilia were much more tightly packed than normal. The length of the cilia was estimated to be about 4.3 microns. The nuclei of the ciliary cells seemed more oval and elongated than those in the unexposed rats.

In many light micrographs the entire epithelium seemed to be lifted up from the lamina propria. Whether the cause was oedema or an artefact could not be determined with certainty. Most of the exposed rats, however, showed this change, which was not found to the same extent in the healthy rats. Within the lamina the collagen fibrils in some regions were split and fragmented. The capillaries were dilated. In most cases there was oedema 10 to 20 microns in depth and perivascular extravasation of blood. These changes were seen in both groups of exposed rats. Figure 35 shows the light micrograph from a rat in group 1 and Figure 36 is from a rat in group 2.

Although oedema of the lamina propria was seen in most of the exposed rats, the elongation of the cells and crypt formation could occur in the absence of such oedema. This is illustrated in Figure 37, a micrograph from a rat in group 2.

In both groups exposed to sulphur dioxide three rats, as earlier stated, were not examined until 33 days after the exposure was terminated. Light microscopy of the tracheal epithelium then showed changes the same as those described above. Figures 38 and 39 derive from one rat in each group.

*Control rats exposed only to the air inflow of the exposure chamber* displayed no changes in the tracheal epithelium as compared with healthy rats.

#### **Electron microscopy**

The findings at light microscopy were also apparent in the electron micrographs. Thus the ciliary cells were elongated and slender with compressed nuclei (Figures 40 and 41).

The surface of the epithelium displayed deep crypts which were packed with cilia. Figure 42 shows such a crypt from a rat in group 1. A pronounced accumulation of cilia is seen in Figure 43, from a rat belonging to group 2. In addition, very many bacteria, as described by Birch-Andersen, Maaløe and Sjöstrand (1953, 1954), are present among the cilia (Figures 43 and 44). The number of cilia per square micron was not determined. Nor did this appear necessary, as the micrographs from all the exposed rats clearly showed the cilia to be more tightly packed than normal on the cell surface.



*Figure 32.* Tracheal epithelium from a rat exposed for 18 days to 11.4 p.p.m.  $\text{SO}_2$ . The ciliary cells and their nuclei appear compressed. Deep crypts are present in the epithelium and are packed with cilia. Phase contrast. Magnification 2,180  $\times$ .



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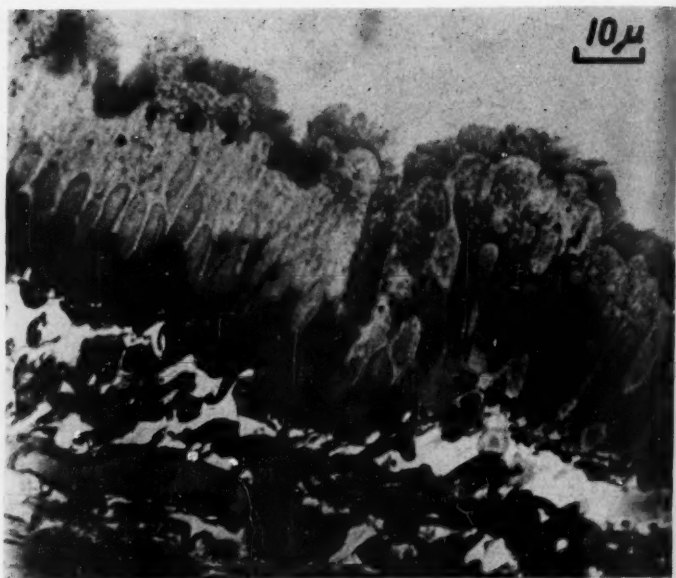
Figure 34. Tracheal epithelium from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ , showing the same changes as in figure 33. Phase contrast. Magnification  $2,010\times$ .

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Figure 35. Longitudinal section of tracheal epithelium from a rat exposed for 18 days to 11.4 p.p.m.  $\text{SO}_2$ . The lamina propria shows oedema and collagen fibrils. Beneath these is a dilated blood vessel. Phase contrast. Magnification 2,107  $\times$ .



*Figure 36.* Longitudinal section of tracheal epithelium from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ . The entire epithelium appears to float on the oedema. A dilated blood vessel is seen.  
Phase contrast. Magnification  $960\times$ .



*Figure 37.* Longitudinal section of tracheal epithelium from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ . A number of goblet cells is seen. No definite oedema is discernible, although the epithelium otherwise shows the same changes as in figures 33 & 34. Phase contrast. Magnification  $2,070\times$ .



Figure 38. Longitudinal section of rat tracheal epithelium 33 days after termination of exposure (for 18 days) to 11.4 p.p.m.  $\text{SO}_2$ . The changes are analogous with those in figures 35 and 36. The cells are slender, some of them are goblet cells and interspersed goblet cells, some of them are loaded with mucus.

Figure 38. Longitudinal section of rat tracheal epithelium 33 days after termination of exposure (for 18 days) to 11.4 p.p.m.  $\text{SO}_2$ . The changes are analogous with those in figures 33 & 34—deep crypts, slender ciliary cells and interspersed goblet cells, some of them filled with mucus.



Figure 39. Longitudinal section of rat tracheal epithelium 33 days after termination of exposure (for 67 days) to 11.5 p.p.m.  $\text{SO}_2$ . The changes are analogous with those in figures 33 & 34—crypt formation in the epithelium and a number of goblet cells, some filled with mucus.

Phase contrast. Magnification 3,760  $\times$ .



*Figure 40.* Longitudinal section of ciliary cells from a rat exposed for 18 days to 11.4 p.p.m.  $\text{SO}_2$ . The ciliary cells are elongated and slender. A goblet cell with mucous granules is seen in a crypt.  
Electron micrograph. Magnification 8,300  $\times$ .

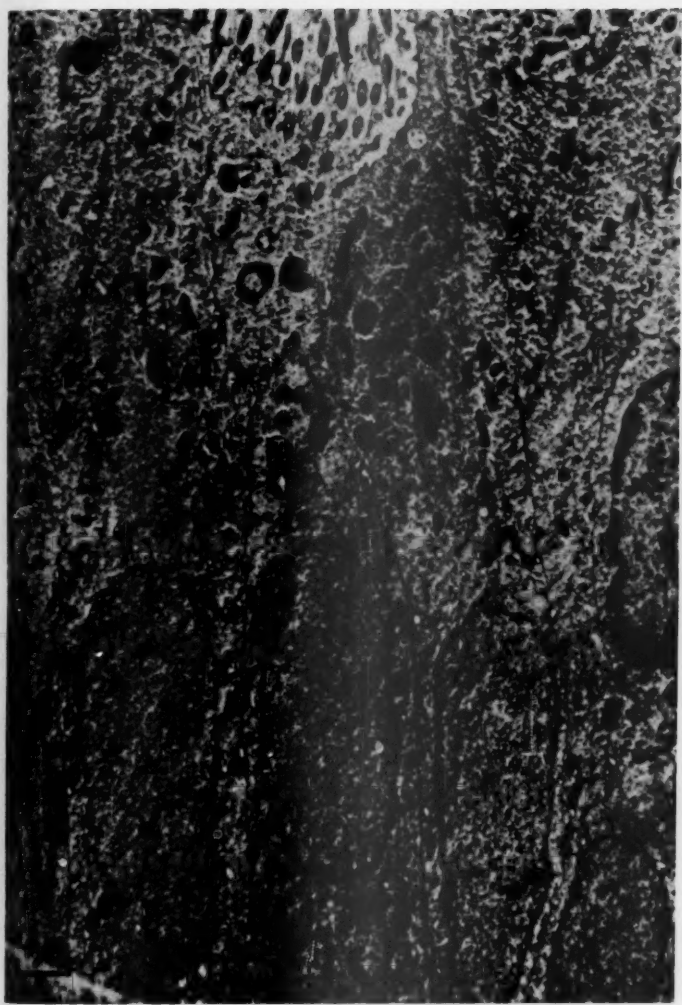


Figure 41. Longitudinal section of ciliary cells from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ . The cells are elongated. The nuclei also appear longer than normal. At the top of the micrograph the base of a crypt is visible.  
Electron micrograph. Magnification  $8,300\times$ .



*Figure 42.* Deep crypts with tightly packed cilia in a rat exposed for 18 days to 11.4 p.p.m.  $\text{SO}_2$ . The peripheral and central filaments of the cilia are clearly distinguishable.

Electron micrograph. Magnification  $24,200 \times$ .

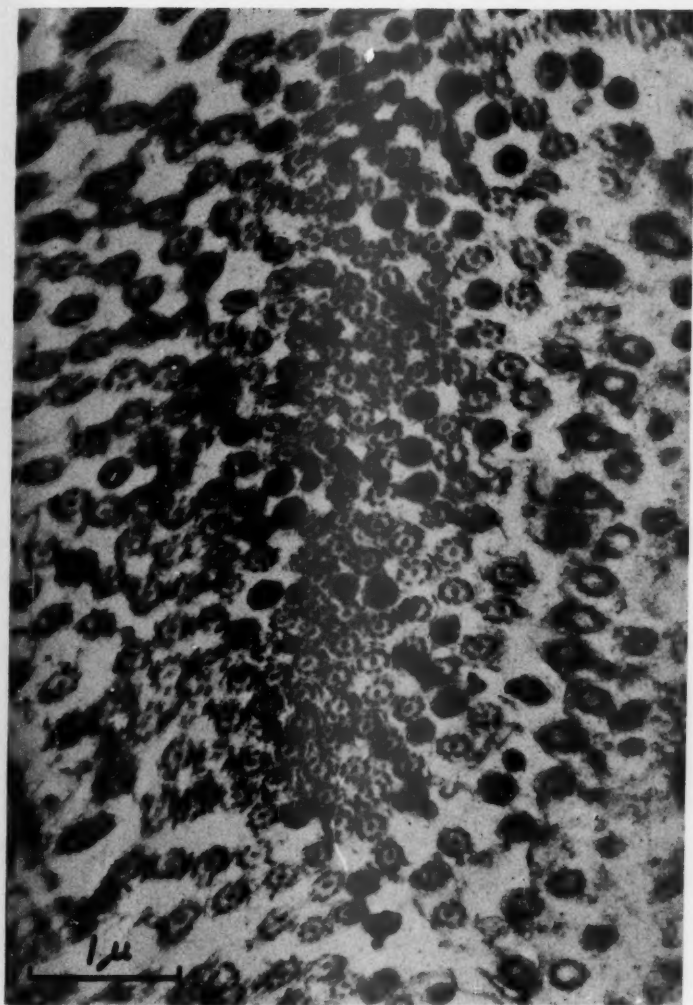
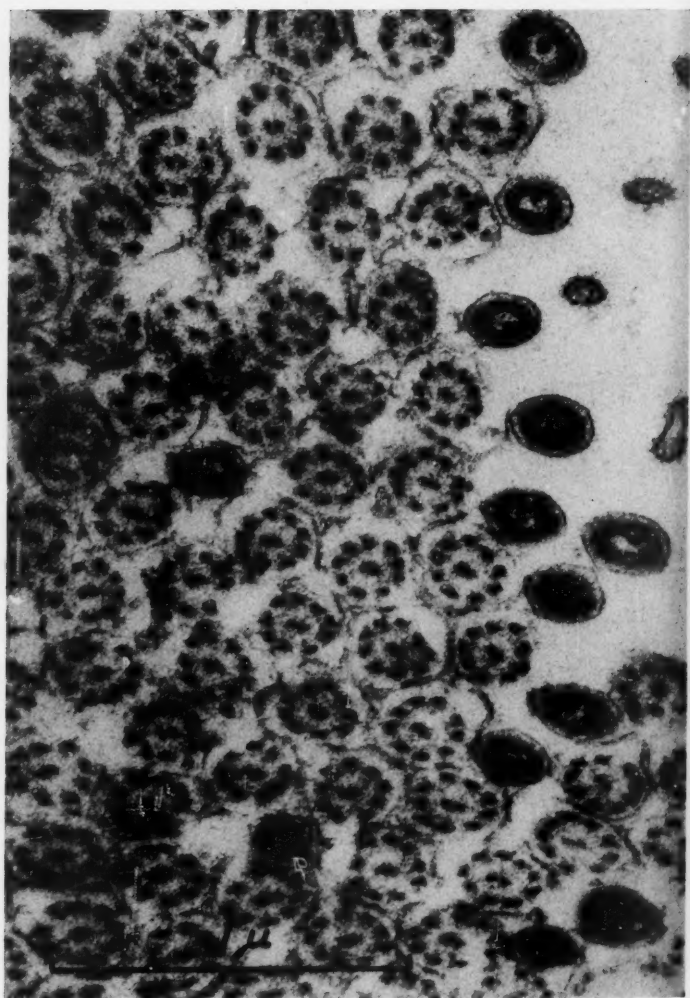
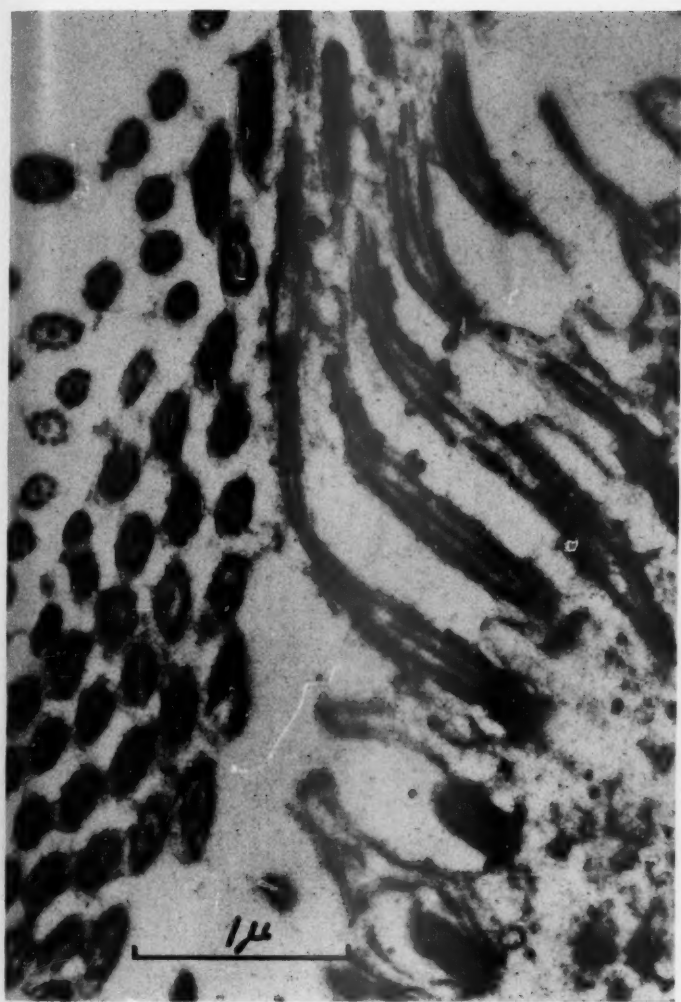


Figure 43. An epithelial crypt in a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ .  
Between the cilia are many bacteria.  
Electron micrograph. Magnification 22,900  $\times$ .



*Figure 44.* Dense accumulation of cilia in an epithelial crypt and profuse bacteria.  
From a rat exposed to 11.5 p.p.m.  $\text{SO}_2$  for 67 days.  
Electron micrograph. Magnification  $54,300\times$ .



*Figure 45.* Transverse and longitudinal sections of cilia from a rat exposed for 18 days to 11.4 p.p.m.  $\text{SO}_2$ . The peripheral and central filaments are clearly distinguishable. The former may be followed down into the basal corpuscles. Electron micrograph. Magnification  $32,500\times$ .



*Figure 46.* Longitudinal section of a cilium from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ , showing the outer sheath and the tubelike peripheral and central filaments. At the top of the figure is a cross section of a cilium.  
Electron micrograph. Magnification  $51,000\times$ .

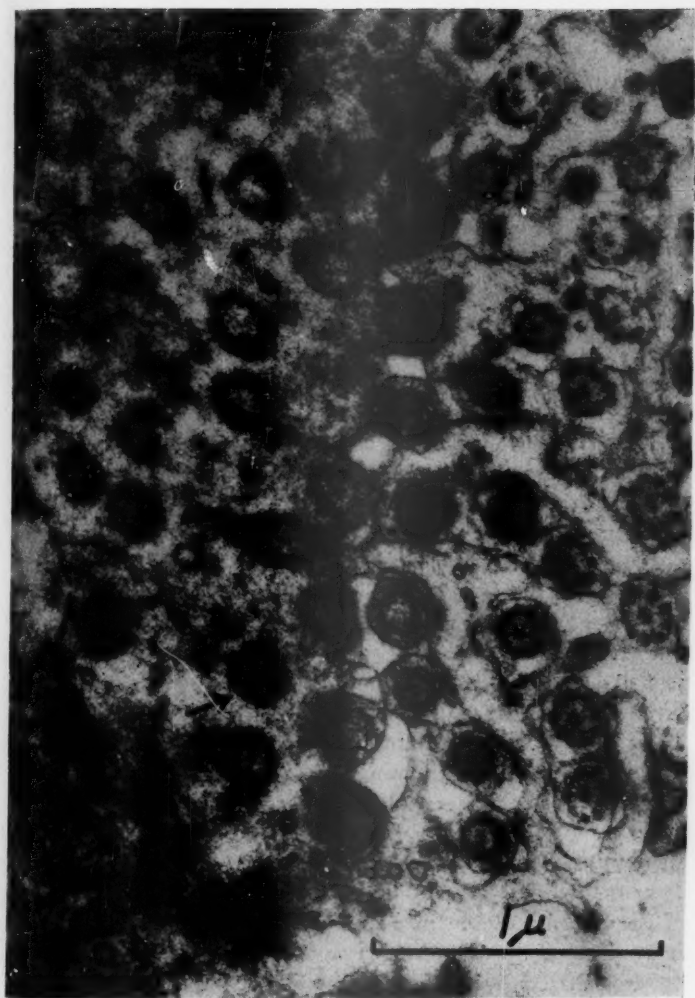


Figure 47. Transverse sections of cilia at various levels from a rat exposed for 67 days to 11.5 p.p.m.  $\text{SO}_2$ . As in figure 27, the peripheral filaments gradually converge and finally form a raspberry-like pattern. The transversely cut kidney- or bean-shaped body is also visible (arrow).  
Electron micrograph. Magnification  $43,400\times$ .

The ultrastructure of the cilia was unaltered in comparison with the unexposed rats. Figure 45 shows longitudinally and transversely cut cilia from a rat in group 1. The thickness of the peripheral and central filaments was about 300 Å. Figures 46 and 47 present a vertical and a transverse section from a rat in group 2. In the latter micrograph the mutual relationship of the filaments is seen to be the same as in unexposed rats.

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The *electron microscopy of healthy rats* confirmed the findings of Fawcett and Porter (1954) concerning Fallopian tube cilia. Some new structural details, however, were revealed by the present study of tracheal cilia. Whether or not these observations are characteristic of, and limited to, the tracheal cilia cannot as yet be determined. The nine peripheral filaments were thus seen to be double, with a common partitioning membrane. Further, fusion of the peripheral filaments in the extreme tip of the cilium may be considered probable and would explain the micrographs obtained by Engström and Wersäll (1952). In the basal corpuscle a kidney- or bean-shaped body was detected.

Microscopic examination of *rats exposed to sulphur dioxide* showed that about 10 p.p.m. for approximately 3 or 10 weeks produced severe morphologic changes of the epithelium and lamina propria. These changes were unaffected by the difference in the duration of exposure. Nor did they appear to have regressed in the rats first examined about 4 weeks after exposure to  $\text{SO}_2$  had ceased. This latter group, however, comprising 3 rats from each of groups 1 and 2, was too small to permit definite conclusions concerning regression from the morphologic studies alone.

The packing of the cells and the crypt formation may have arisen from cell proliferation, from the epithelium being pushed in towards the tracheal lumen by the underlying oedema, or from a combination of both factors.

As the depth of the oedema, however, was only 10 to 20 microns, and as at least 500 microns would have been required to produce the observed compression of the cells, the probable cause of this compression was cellular proliferation. The increased density of

the cilia in the exposed rats was probably due to the diminished surface of the compressed cells. There were no grounds for presuming that ciliary proliferation took place.

Electron microscopy failed to demonstrate any alteration of ciliary ultrastructure after exposure to  $\text{SO}_2$ . It appeared, therefore, that the cilia remained undamaged by this exposure although the surrounding tissues, including the ciliary cells, showed severe changes. This finding tallies well with the general impression that the cilia are highly resistant structures.

## General Discussion

The experimental findings presented in the foregoing chapters have already been discussed in their respective connections. A summarized discussion of the most important results is nevertheless appropriate for the elucidation of certain points.

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It was of great potential value that the experiments should permit conclusions valid for corresponding conditions in man. Therefore it was desirable that the animals studied should be mammals and that the conditions of the experiments should approach as closely as possible the physiologic environment in the trachea of the intact animal. It was also important that the methods used for determining the speed of mucous flow and the frequency of ciliary beat should have the smallest possible experimental error. Under such circumstances the interpretation of findings after exposing the animals to some form of treatment would not be hampered by the method as such.

These requirements for the study of mucous transportation and ciliary activity were largely fulfilled by the writer's method. The experiments were thus carried out on living rats. Although the physiologic conditions, naturally, were not identical with those in the intact animal, they may be regarded as satisfactory. The rats, for instance, could lie in the moist chamber for a comparatively long time without showing changes in the functions under investigation.

The writer's method permits accurate standardization. It is strongly stressed, however, that such standardization as regards ciliary beat

frequency should be carried out by a method of counting similar to that described on page 38.

The fact that certain variations occurred in the counts of different workers shows that the experimental error in this study is not routinely applicable. For each investigation the ciliary movement should be calculated within different frequency ranges and the results should be verifiable by other workers.

Larger animals such as dogs, cats and rabbits are not suitable for study with the writer's technique. The values registered in the rats cannot unreservedly be applied to other mammals. From the literature it would seem that ciliary activity and mucous flow do not proceed at the same rates in different mammals. The feasibility of enlarging the moist chamber sufficiently to permit similar studies of larger animals does not seem to be great since, *inter alia*, the temperature in the chamber would then be considerably more difficult to regulate. If determination of the ciliary beat frequency only is desired, however, the writer's method may be useful for *in vitro* studies, as no definite alteration of ciliary activity could be demonstrated for some time after extirpation of the trachea. In this way the scope of the method could be considerably extended.

It would therefore seem that the method evolved by the writer, and used for determining the mucous flow and ciliary beat rates in the trachea, has great advantages in comparison with earlier-described methods.

The possibilities of standardizing the method render it suitable for studying the action of various agents on the mucous flow and ciliary activity in the trachea. Investigations of the effect of various gases and of locally and orally administered drugs may thus be made considerably more comprehensive. An interesting aspect is the feasibility of placing laboratory animals in an industrial environment, the influence of which on the upper respiratory tract may then be studied. The findings from such studies should assist the assessment of health hazards. It is finally probable that the writer's technique is applicable to other ciliated membranes than the respiratory mucosa. Determination of the frequency of ciliary beat in, for instance, the Fallopian tubes should be within the scope of the method.

In the healthy rats elevation of the rectal temperature up to a certain limit entailed acceleration of the mucous flow. In conditions such as febrile infections of the respiratory tract this acceleration must be considered important for hastening excretion of the bacteria in the mucus.

When both the chamber temperature and the rectal temperature were lowered the speed of mucous transportation and ciliary activity diminished, but increased when the temperatures were normalized.

It is presumed that one cause of these changes in the velocity of mucous flow may have been the observed variation in the frequency of ciliary beat. Other factors, however, such as altered composition or quantity of the secretion, also played a part.

In the rats subjected to protracted exposure to sulphur dioxide there was retardation of the mucous flow and in some cases also of the ciliary beat. One cause of the slowing of mucous flow in the rats with normal ciliary activity probably was the directly demonstrated increase in the amount of secretion.

The findings in the exposed rats were supported by the morphologic studies of the tracheal mucosa. No change in the ciliary structure was seen. The epithelium showed other changes, however. Prominent among these was the very irregular epithelial surface with deep crypts produced by cell proliferation. Since the cilia lining the crypts could not contribute to the transportation of mucus, such changes were a probable cause of the retarded mucous flow. It therefore seemed that the damage caused by exposure to  $\text{SO}_2$  did not primarily affect the cilia and their movement mechanism, but rather the secretory component of mucosal function. The epithelial changes presumably arose from direct action on the epithelium of the gas dissolved in the secretion. It is also pertinent to point out that the changes were seen after exposure to  $\text{SO}_2$  in a concentration which, according to general conceptions of industrial health, can be tolerated during an eight-hour working day. This concentration, therefore, should be subjected to renewed evaluation, as the consequences of the above-mentioned disturbances in epithelial function are of great clinical importance.

The investigations of how the tracheal epithelium reacts to single exposure to irritant gases showed that ciliary movement ceases after a period determined by the gas employed, its concentration and solubility in water. They also demonstrated, however, that ciliary beating, and therewith mucous transportation, rapidly recommence when such exposure is terminated. If the exposure is repeated during a fairly long period, the secretory mechanism of the mucosa reacts while the ciliary mechanism in some cases may remain intact. This emphasizes the opinion advanced by the writer, that the ciliary mechanism is less easily influenced than mucous transportation. Although there are no grounds for assuming that the described mode of mucosal reaction in rats differs from that in man under comparable conditions, the writer's experiments do not permit definite conclusions concerning the action on the human respiratory tract of industrial exposure to gases.

## Summary

The main object of this study was to evolve a *method* with which the speed of mucous transportation and the frequency of ciliary beat could be determined in the trachea of living mammals. *Normal values* for the functions in question were determined in rats. The influence of *certain physiologic factors* on these values was investigated. The usefulness of the method for evaluating function changes in certain *pathologic conditions* was shown in experiments with respiratory irritant gases. *Morphologic studies* with light microscopy and electron microscopy were carried out parallel with some of the investigations of function.

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The principle of the *method* was that anaesthetized rats with the trachea opened were placed in a specially-constructed chamber, in which the humidity and temperature of the air could be regulated. A microscope mounted on the chamber permitted direct inspection of the tracheal mucosa. The details of the method were planned with the aim of conserving intact the physiologic environment of the tracheal mucosa for as long as possible, while being able to effect controlled variations of factors which might influence mucous transportation and ciliary activity. These two functions could thus be investigated under conditions practically constant as regards humidity and temperature and without the assistance of foreign substances such as saline solutions and artificial indicators of mucous flow. In this way determinations of the mucous flow and ciliary beat rates could be carried out with an experimental error which by and large did not exceed 10 per cent.

The rate of mucous flow was calculated by measuring the time taken for particles such as shed epithelial cells, which had become fixed in the tracheal secretion, to travel a known distance. The frequency of ciliary beat was determined by cinematographic recording with a camera speed of 128 exposures per second and subsequent projection of the film. The time relationship between the rapid and the slower phases of the single ciliary beat was studied on films taken with a speed of 500 to 700 exposures per second.

In the determinations of ciliary beat frequency, individual variations were shown at high frequencies in the counts of different assistants. Conclusions concerning the rapid rates of ciliary beat should therefore be cautiously made. For present purposes, however, this circumstance is of minor importance, as the rate of ciliary beat following exposure to respiratory irritant gases may *a priori* be assumed to be unaltered or reduced. Nevertheless in the only group of rats which showed acceleration of the ciliary beat, the rising tendency was recorded by three different assistants.

In a "normal material" comprising 37 white male rats the mean velocity of mucous flow was 13.5 mm per minute and the mean frequency of ciliary beat 1,317 beats per minute when the air in the observation chamber was saturated with water vapour and had a temperature of 34° C. The standard deviation in regard to the differences between the individual rats was 22.3 per cent of the mean for mucous flow and 13.4 per cent of the mean for ciliary beat. The rapid phase of ciliary beat was found to occupy about one third of the time required by the slower phase.

In comparative *in vivo* and *in vitro* experiments it was found that mucous transportation ceased within a relatively short time after the trachea was extirpated. The ciliary activity also diminished, although this occurred later than the effect on mucous transportation. These findings imply that the *in vitro* method may be useful for studying the rate of ciliary beat, provided a sufficiently rapid preparation technique can be evolved.

It was demonstrated that changes in *certain physiologic factors*, viz. the humidity and temperature of the air and the temperature of the tracheal tissues, could greatly affect the functions of the mucosa.

Thus reduction of the relative atmospheric humidity could very quickly bring about cessation of ciliary activity.

Elevation of the chamber temperature, on the other hand, did not give rise to major variations in either mucous flow or ciliary activity.

Elevation of the tissue temperature with unaltered chamber temperature resulted in acceleration of mucous transportation up to a temperature of 39° C. At higher tissue temperatures slowing of the mucous flow was recorded. The ciliary activity, on the other hand, showed no significant change.

Elevation of both the chamber temperature and the rectal temperature entailed significant increase of the rate of mucous flow up to a rectal temperature of 39° C. At higher temperatures deceleration of this function occurred. The rate of ciliary beat also showed a clear tendency to increase.

Reduction of the chamber temperature and the rectal temperature to 25.2° and 31.2° C, respectively, resulted in significant deceleration of both mucous transportation and ciliary activity. When the temperatures were raised to the readings employed in the normal rats, however, the functions in question returned to almost normal values.

For investigation of the influence of experimental duration the rats were allowed to lie in the chamber for 120 minutes and determinations of the mucous flow and ciliary beat rates were made at intervals. Mucous transportation was unaltered 80 minutes after the trachea was opened, but had become affected after 120 minutes. Ciliary activity showed no change even after 120 minutes.

Experiments were also made to apply the method to *pathologic conditions*. Rats were exposed to about 10 p.p.m. sulphur dioxide for 67 days or 18 days. The mucous flow and ciliary activity were then examined as described for healthy rats. In several rats from both exposed groups there was no measurable transportation of mucus or the rate of flow was reduced. The ciliary activity, on the other hand, appeared unchanged in one group, but was reduced in the other. Three rats exposed to SO<sub>2</sub> for 18 days and three exposed for 67 days were not examined until about one month after the cessation of exposure. In these animals, too, the transportation of mucus was retarded or absent. In one group deceleration of ciliary movement was recorded. The time ratio between the effective and

recovery strokes of the ciliary cycle was studied in a few of the exposed rats. No change in this relationship was found. As the changes after exposure to  $\text{SO}_2$  could conceivably have been due to *inter alia* quantitative alteration of the secretion, the thickness of the mucous blanket in healthy rats and a rat exposed to  $\text{SO}_2$  was determined with a histologic technique. Even to naked-eye observation it was obvious that the mucous blanket was considerably thicker in the exposed rat. The blanket measured about 5 microns in the healthy rats, but about 25 microns in the exposed rat.

Single exposure to ammonia, formaldehyde and sulphur dioxide resulted in cessation of ciliary movement after a varying time from the commencement of exposure. This interval appeared to be dependent on the type of gas used and its solubility in water. Thus the cilia ceased beating after 20 seconds in rats exposed to 20 p.p.m. ammonia, after about 10 seconds' exposure to 22 p.p.m. formaldehyde and after about 2 minutes with 25 p.p.m. sulphur dioxide. When the exposure was terminated, however, ciliary activity very rapidly recommenced—after 10 to 30 seconds. Rats previously exposed to 10.4 p.p.m. sulphur dioxide for 48 days showed the same mode of reaction to single  $\text{SO}_2$  exposure as previously unexposed rats. Thus there was no evidence of habituation to the effects of the gas. Nor did there seem to be any change in the pH of the secretion following protracted exposure to  $\text{SO}_2$ , or at least none which could be demonstrated with the method employed.

The *morphologic studies* of the tracheal epithelium in healthy rats revealed some structural details hitherto undescribed. Electron microscopy showed that the cilia, of which there are about 8 per square micron, consist of two central and nine peripheral filaments. These latter appear to fuse at the tip of the cilium. Towards the base of the cilium the peripheral filaments converge to form a wreath-like structure which encloses a weakly osmiophilic substance. Within this substance an opaque, kidney- or bean-shaped body was observed. Ciliary rootlets could be traced into the cell only for about 0.5 micron. In addition to the ciliary cells and goblet cells, the epithelium was seen to contain a type of cell with protoplasmic projections. Such cells were designated "brush cells".

The morphologic studies of rats subjected to protracted  $\text{SO}_2$

exposure gave results which tallied well with those concerning function. The surface of the epithelium was very irregular with deep crypts in places and compressed ciliary cells. Beneath the epithelium were changes which probably corresponded to oedema with vascularization and with split and fragmented collagen fibrils. These findings were confirmed by ultrastructural studies. The ciliary structure, on the other hand, appeared unchanged.

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From the results of this study it would seem that the method evolved by the writer is well suited for studying the velocity of mucous flow and the frequency of ciliary beat in the trachea of living rats. The method was shown to be applicable in certain pathologic states. It is probably also useful for studying other ciliated organs such as the Fallopian tubes. Determination of the effect on mucous flow and ciliary activity of different substances administered orally, locally or parenterally is likewise feasible.

It was further found that temperature plays a considerable role in the maintenance of normal mucous flow and ciliary activity. Concurrent elevation of the temperature of the environmental air and the tissue temperature entailed acceleration of both functions.

Protracted exposure to about 10 p.p.m.  $\text{SO}_2$  resulted in retardation of the mucous flow and in some cases also of the ciliary activity. This slowing of the mucous flow was due to *inter alia* the increased thickness of the mucous blanket seen in the exposed rats and the morphologically-demonstrated epithelial crypts.

## Sammanfattning

Den huvudsakliga uppgiften i detta arbete har varit att utarbeta en metod för in-vivo-bestämning av sekrettransport och flimmerfrekvens i trachea hos däggdjur. Med denna metod har sedan bestämts *normalvärden* för de nämnda funktionerna hos råtta samt undersökts hur dessa normalvärden påverkas av *vissa fysiologiska faktorer*. Metodikens användbarhet för undersökningar av funktionella förändringar vid *vissa patologiska tillstånd* har visats i expositions försök med lungretande gaser. Parallellt med de funktionella studierna ha i viss omfattning utförts *morfologiska undersökningar* med ljus- och elektronmikroskopi.

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*Metoden* har principiellt bestått i att en narkotiserad råtta med uppklippt trachea placerats i en specialkonstruerad kammare där luftens fuktighet och temperatur kunnat kontrolleras. Ett till kammaren anslutet mikroskop har medgivit en direkt inspektion av slemhinnan. Detaljerna i metodiken har utarbetats med tanke på att så långt som möjligt kunna hålla trachealslemhinnans fysiologiska miljö intakt, resp. att kunna under kontroll variera sådana faktorer som väntas kunna påverka de studerade funktionerna. Undersökningen av sekrettransporten och flimmeraktiviteten har således kunnat ske under i det närmaste konstanta fuktighets- och temperaturförhållanden och med undvikande av varje slag av miljöfrämmande hjälpmedel såsom saltlösningar och artificiella transportindikatorer. Metodiken har på detta sätt medgivit bestämningar av transporthastighet och flimmerfrekvens med ett experimentellt fel som i stort sett icke överstigit 10 %.

Sekretets transporthastighet har bestämts genom avläsning av den tid det tagit för vissa i sekret inbakade partiklar att vandra en känd vägsträcka. Flimmerfrekvensen har erhållits genom filmning med 128 bilder/sek. och efterföljande projektion av filmen. Vid filmning med mycket hög filmhastighet, 500—700 bilder/sek., har det även varit möjligt att bestämma förhållandet mellan den snabba och den långsamma fasen i det enskilda cilieslaget. Vid bestämning av flimmerfrekvensen har det visat sig att individuella skiljaktigheter hos olika personer gör sig gällande vid höga frekvenser. Bedömningen av höga frekvenser bör därför alltid ske med försiktighet. I föreliggande arbete har emellertid denna omständighet varit av mindre intresse då effekten på flimmerfrekvensen kan antagas endera lämna frekvensen intakt eller minska den. I den enda grupp där en tendens till stegring av flimmerfrekvensen kunnat konstateras har emellertid denna tendens kunnat verifieras av tre olika räknare.

I ett »normalmaterial» omfattande 37 vita hanrättor har vid mättad luftfuktighet i kammaren och vid en lufttemperatur av 34° sekretets transporthastighet bestämts till 13,5 mm/min. och flimmerfrekvensen till 1 317 slag/min. Standardavvikelsen med avseende på försöksdjurens individuella olikheter uppgick till 22,3 % och 13,4 % av resp. medeltal. Förhållandet mellan den snabba och den långsamma fasen i det enskilda cilieslaget har bestämts till ca 1 : 3.

Vid de jämförande *in vivo* — *in vitro* försök som utförts har det visat sig att sekrettransporten relativt hastigt upphörde efter extirpation av trachean. Även flimmerfrekvensen minskade ehuru denna effekt ur tidssynpunkt kom senare än motsvarande effekt på sekrettransporten. Detta innebär att *in vitro* försök kan tänkas komma till användning för bestämning av flimmerfrekvensen under förutsättning att en tillräckligt snabb preparationsmetodik har utarbetats.

I samband med metodikstudierna har författaren även kunnat visa att förändringar av vissa fysiologiska förhållanden såsom av luftens fuktighet och temperatur samt av vävnadens temperatur påverkar trachealslemhinnans funktion.

Sålunda har det visat sig att en minskning av luftens relativa fuktighet mycket snabbt kan leda till att all flimmeraktivitet upphör.

En förhöjning av kammarens temperatur synes däremot icke föranleda några större variationer i vare sig sekrettransport eller flimmerfrekvens vid bibehållen konstant rectaltemperatur.

Höjning enbart av vävnadens temperatur och vid bibehållen kammartemperatur resulterade i en ökning av transporthastigheten upp till 39° varefter ytterligare höjning medförde en minskning av transporthastigheten. Någon säkerställd effekt på flimmerfrekvensen kunde däremot icke iakttagas.

Vid höjning av såväl kammartemperatur som rectaltemperatur erhöles en säkerställd höjning av transporthastigheten upp till en rectaltemperatur av 39° varefter ytterligare höjning medförde en minskning. Flimmerfrekvensen visade även en klar tendens till ökning.

En sänkning av både kammartemperatur och rectaltemperatur till 25,2° resp. 31,2° resulterade i en uttalad sänkning av både sekrettransport och flimmerfrekvens. När temperaturerna åter höjdes till de värden vilka använts i normalmaterialat återtog funktionerna i det närmaste normala värden.

För att undersöka vilken effekt som övriga försöksbetingelser hade på djuren fingo dessa ligga 120 min. i kammaren med intervallvis bestämning av sekrettransport och flimmerfrekvens. Det visade sig härvid att sekrettransporten icke visade någon förändring 80 min. men väl 120 min. efter det att trachean öppnats. Flimmerfrekvensen uppvisade däremot icke ens efter 120 min. någon förändring.

Försök att tillämpa metodiken på *patologiskt material* har gjorts. Författaren har sålunda exponerat råttor för omkr. 10 p.p.m. SO<sub>2</sub> under olika perioder, 67 dagar och 18 dagar. Därefter har på ovan beskrivet sätt sekrettransport och flimmerfrekvens undersökts. Det visade sig härvid att hos ett flertal av djuren ur båda de exponerade grupperna sekrettransporten ej alls gick eller var nedsatt. Någon förändring av flimmerfrekvensen kunde däremot icke konstateras i en grupp medan aktiviteten däremot var nedsatt i den andra. Ur de båda exponerade grupperna hade tre djur ur vardera icke undersökts förrän ca 1 månad efter den sista exponeringen. Även bland dessa djur var sekrettransporten nedsatt eller helt avstannad. I en av grupperna kunde dessutom en nedsättning av flimmerfrekvensen iakttagas. På ett fåtal exponerade djur har även undersökts huruvida

någon förändring av förhållandet mellan "effective and recovery" stroke uppkommit. Någon dylik förändring kunde emellertid icke konstateras. Då de erhållna resultaten bl. a. kunna tänkas bero på förändringar i sekretets mängd har författaren med hjälp av histologisk teknik bestämt sekretsiktets tjocklek hos friska och exponerade djur. Redan för blotta ögat var det tydligt att sekret siktets tjocklek väsentligt ökats hos de exponerade djuren och vid uppmätning av skikttjockleken befanns den hos normala djur vara omkr.  $5\mu$  medan den hos ett exponerat djur var omkr.  $25\mu$ .

Kortvarig exposition för ammoniak, formaldehyd och svaveldioxid har medfört att flimmeraktiviteten upphört kortare eller längre tid efter exponeringens början. Denna tid synes beroende av såväl vilken gas som använts som gasens vattenlöslighet. Sålunda avstannade cilieaktiviteten efter 20 sek. vid exposition för 20 p.p.m. ammoniak, efter ca 10 sek. för 22 p.p.m. formaldehyd och efter ca 2 min. för 25 p.p.m. svaveldioxid. Efter exponeringens upphörande återkom emellertid cilieaktiviteten mycket snabbt efter 10—30 sek. Djur vilka exponerats för 10,4 p.p.m.  $\text{SO}_2$  under 48 dagar visade vid akut exponering inga skillnader gentemot de förut icke exponerade med avseende på flimmeraktivitetens reaktionssätt. Någon tillvänjning för svaveldioxid synes sålunda icke ha uppkommit. Ej heller synes någon förändring av sekretets pH efter långvarig exposition för svaveldioxid ha uppkommit eller i varje fall icke kunnat påvisas med den av författaren använda metodiken.

Vid den morfologiska undersökningen av tracheans epitel hos friska djur kunde några hittills icke beskrivna detaljer iakttagas. Elektron mikroskopi visade att cilier, vilka finnas till ett antal av omkr.  $8/\mu^2$  består av två centrala och nio perifera filament. De senare synas förena sig i topparna på cilien. Mot ciliens bas flyter de perifera filamenten samman till en kransliknande struktur vilken innesluter en svagt osmiophil substans. I denna har observerats en opaque njur- eller bönformad bildning. «Ciliary rootlets» har endast kunnat följas in i cellen till omkr.  $0,5\mu$ . Förutom de observerade ciliecellerna och bägarcellerna har i epitelet kunnat iakttagas en typ av celler med protoplasma utskott. Dylika celler ha här kallats »brush cells».

Den morfologiska undersökningen av de under längre tid  $\text{SO}_2$ -exponerade råttorna har givit resultat vilka väl korrespondera med de funktionella fynden. Epitelytan var mycket oregelbunden med

ställvis djupa kryptor och hoptryckta cilieceller. Under epitelet syntes förändringar vilka med sannolikhet härröra från ödem, vaskularisering och uppsplittrade och fragmenterade kollagena fibriller. Dessa fynd bekräftas av den ultrastrukturella undersökningen. Ciliestrukturen visade sig å andra sidan oförändrad.

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Av undersökningsresultaten att döma synes den av författaren utarbetade metodiken vara väl ägnad för bestämning av sekrettransport och flimmerfrekvens i trachean på levande råttor. Att metoden är tillämplig på vissa patologiska förhållanden har även visats. Det är icke otänkbart att andra undersökningsobjekt såsom tuba Fallopi och andra organ med flimmerepitel kunna undersökas med den beskrivna metoden. Bestämning av inverkan på sekrettransport och flimmerfrekvens av olika farmaka endera tillförda per os, lokalt eller parenteralt kunna även utföras.

Av undersökningsresultaten synes vidare framgå att vissa temperaturförhållanden spela en stor roll för sekrettransporten och flimmerfrekvensen. En ökning av både den omgivande luftens temperatur och vävnadens temperatur medför sålunda en effektivisering av de båda aktuella funktionerna.

Långvarig exponering för omkr. 10 p.p.m. svaveldioxid medför en nedsättning av sekretets transporthastighet och i vissa fall även av flimmerfrekvensen. Denna nedsättning av transporthastigheten beror bl. a. på den ökning av sekretsiktets tjocklek som uppkommit hos de exponerade djuren jämte den kryptbildning i epitelet vilken kunnat iakttagas morfologiskt. Någon förändring av ciliernas finstruktur har icke uppkommit efter exponeringen.

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